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Review

Approaches to New Parasiticides†

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Abstract: Intensive use of parasiticides has sometimes led to severe resistance in arthropods and helminths of veterinary importance. In the context of the growing awareness of parasitic diseases, this has created a public demand for effective and safe control agents. During the last two decades considerable knowledge in parasite neurophysiology and endocrinology has accumulated which allows the development of new screening procedures and target-site-directed approaches for the discovery of new drugs. The suitability of this strategic approach is discussed on the basis of recent discoveries of new chemical and natural compounds. In particular, target sites such as $GABA_A$ receptors, muscarinic and nicotinic acetylcholine receptors, cuticle synthesis and degradation, ecdysteroid receptors, the calcium release channel and semiochemicals have been selected in order to demonstrate the current approaches to identify new chemical entities, biologically active against nematodes and arthropods.

Key words: parasiticides, research philosophy, natural compounds, ryanodine receptor, acetylcholine receptor, GABA receptor, insect growth regulators, chitin, sclerotization, insect hormones, neuropeptides, repellents

1 INTRODUCTION

The control of arthropods and arthropod-borne diseases is of great importance due to their massive influence on the profitability of the livestock business and the health status of domestic animals. The growing awareness of arthropods as vectors of zoonotic diseases has created a public demand for effective control agents which could be used safely for the treatment of companion animals and/or hygiene pests in the direct environment of human beings (Table 1). The same is true for diseases caused by helminths and their economic impact. In particular, the losses due to nematode damage in the field of animal health and crop protection are of continuing concern for industrial researchers involved in the discovery of new drugs (Table 1). Intensive use of parasiticides has sometimes led to severe resistance against organochlorines, organophosphates, carbamates and pyrethroids as well as benzimidazoles (Table 1).[1,2] New resistance breaking drugs are needed urgently for further treatment of parasitic diseases.

The difficulties and research costs for the discovery of new chemical entities for either crop protection or to control animal health parasites are similar, but the market sizes for arthropodicides and nematicides differ by at least a factor of two in favour of crop protection (US $ ~ 2·5 billion versus ~8 billion). Therefore, traditionally the ectoparasiticidal and, partially, the nematicidal market have been satisfied by reformulation

† One of a collection of papers on various aspects of pest control research contributed by staff of Bayer AG and collated by Dr M. Londershausen and Dr A. Turberg.

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TABLE 1
Major Nematode and Arthropod Parasites of Importance in the Field of Animal Health and Hygiene

Parasite group[a]	*Examples*	*Treatment*[b] *(used predominantly)*	*Occurrence of resistance*[c]	*Disease carried/ damage caused*
Nematoda				
Trichuridae	*Trichuris*	B; A		
Strongyloididae	*Strongyloides*	A; B		
Strongylidae	*Strongylus,*	A; B; P; OP		
	Cyathostomum		B	
Ancylostomatidae	*Ancylostoma, Uncinaria,*	B; A; L; OP; P		In general:
	Bunostomum			cause tissue destruction,
Toxocaridae	*Toxocara*	B; A; L; OP; (P)		compression, dislocation
Dictyocaulidae	*Dictyocaulus*	A; B		of organs and vessels as
Filariidae	*Dirofilaria*	A		well as intoxication and
Trichostrongylidae	*Cooperia, Haemonchus,*		B; L > > A	deprivation of blood
	Ostertagia,	A; B; L		
	Trichostrongylus,			
Oxyuridae	*Oxyuris*	A; B; OP		
Hypobiotic larvae	*Ostertagia, Cooperia*	A		
Acarina				
Ixodidae (hard ticks)				Heart water, blood loss
	Amblyomma,	Am; SP; OP; A		Anaplasmosis,
	Boophilus,	Am; SP; OP; A	SP; OP, Am	Babesiosis, blood loss
	Hyalomma,	Am; SP; OP; A		Ulceration, lameness
	Ixodes,	Am; SP; OP; A		Louping ill, Lyme disease
	Rhipicephalus	Am; SP; OP; A		Filariasis, Theileria
Sarcoptidae/Psoroptidae/	*Chorioptes,*			Mange
Pyroglyphidae (mites)	*Psoroptes,*			Mange
	Sarcoptes,	SP, OP; A		Mange
	Dermatophagoides			House dust allergy
Insecta				
Culicidae (mosquitos)	*Aedes, Anopheles,*	OP; C; SP		Malaria, Filariasis,
	Culex			Arboviruses
Simuliidae (blackflies)	*Simulium*	OP; SP		Onchocerciasis
Phlebotomidae (sandflies)	*Phlebotomus*	OP; SP		Leishmaniasis, Arbovirus
Muscidae	*Haematobia, Musca,*	OP; SP; BPU; V	OP; SP	Anthrax, Tuberculosis
	Stomoxys			Worms, irritation
Calliphoridae	*Calliphora,*		OP; SP	Myiasis
	Callitroga, Chrysomia,			Myiasis
	Lucilia, Sarcophaga,	OP; SP	OP	Myiasis
	Wohlfahrtia			Myiasis
Oestridae/Gasterophilidae	*Dermatobia,*			Irritation symptoms in
(bot/warble flies)	*Gasterophilus,*	A; OP		alimentary canal and
	Hypoderma, Oestrus			head sinuses
Phthiraptera	*Bovicula, Lepikentron,*			Irritation
(biting/sucking lice)	*Linognathus,*	OP; SP; C; A		
	Haematopinus			
Siphonaptera (fleas)	*Ctenocephalides*	OP; C; BPU		Typhus, plague, worms
Blattidae (cockroaches)	*Blatta, Blattella,*	SP; C; OP	(OP; C; SP)	Tuberculosis, anthrax
	Periplaneta			

[a] Order or family:
[b] A = Avermectins/Milbemycins, Am = Amidine, B = Benzimidazoles, L = Levamisol, P = Pyrantel, C = Carbamates, OP = Organophosphates, SP = Synthetic Pyrethroids, BPU = Benzoylphenylureas (IGR), V = Vetrazin (IGR), ( ) = partially,
[c] = Line corresponds to parasite in which severe resistance has been described.

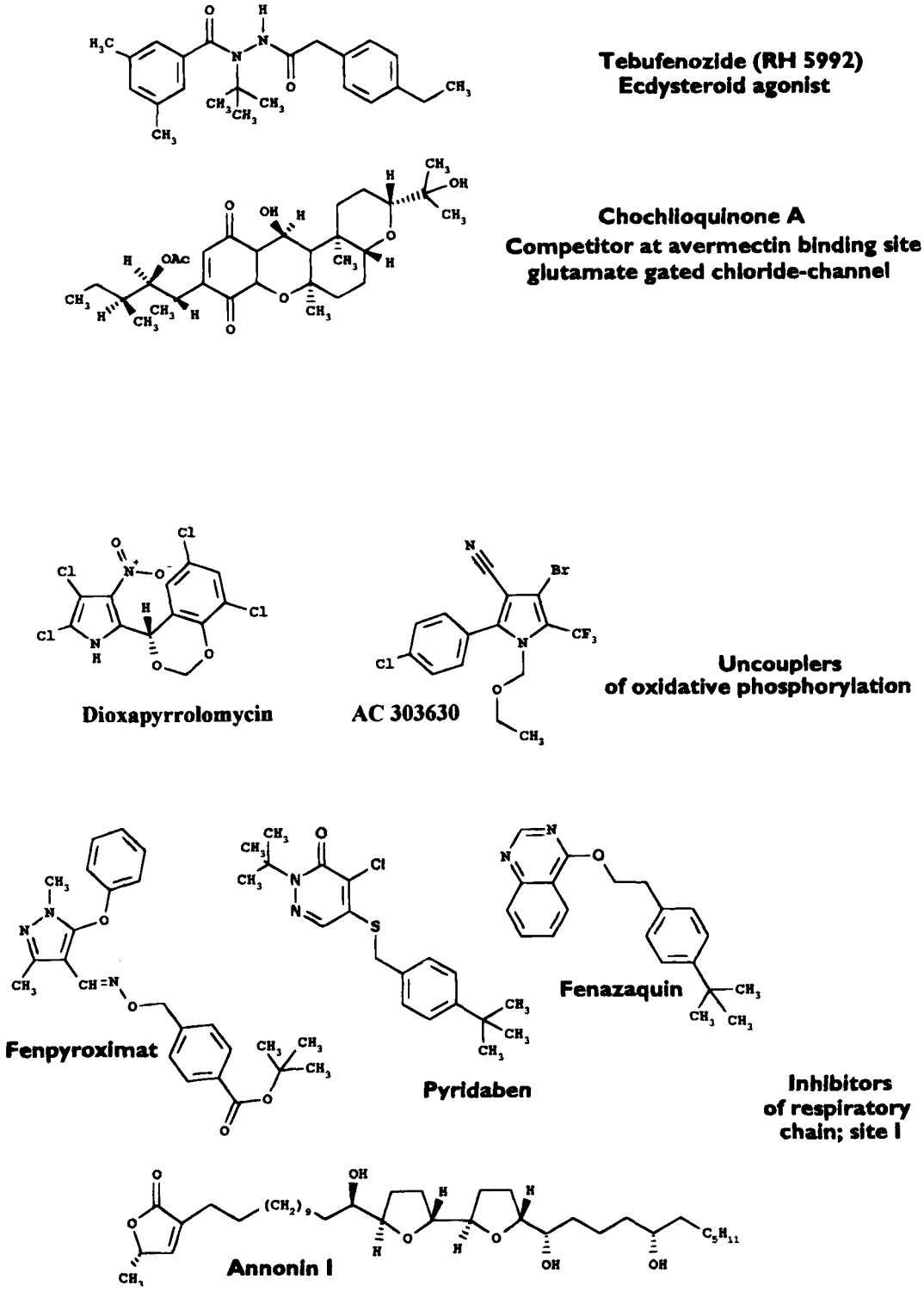
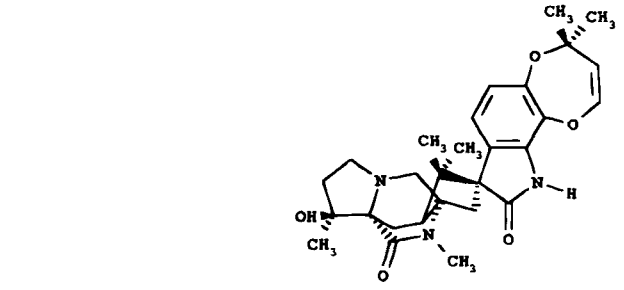
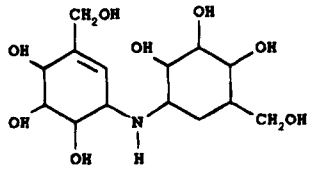


Fig. 1. Various structures affecting target sites of interest for the discovery of new antiparasitic drugs. Various structures from natural origin as well as synthetic compounds are shown which affect different binding sites and display interesting insecticidal or nematocidal activities.

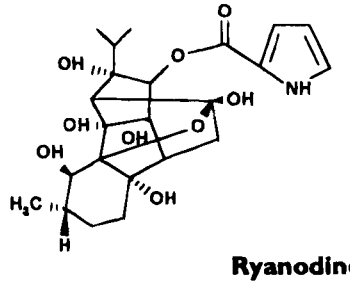
of compounds developed for crop use. However, the chemistry and activity spectrum of these products have increasingly tended not to lend themselves to direct application to animals. For treatment against ticks and mites on livestock, for example, concerns about accumulation of organochlorine residues in milk and meat led to significant limitations of their application. In the case of pyrethroids, cost and structure–activity relationships for the crop protection and animal health derivatives sometimes differed so significantly that separate compounds, such as flumethrin or fenvalerate were developed predominantly for animal health use. The



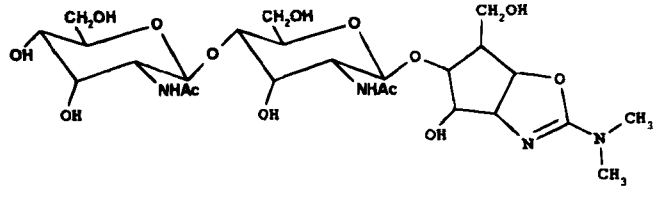
Paraherquamide
Extremely potent competitor at α-BGT binding site (nAChR)
also
Competitor at phenothiazine binding site



Validoxylamine
Inhibitor of insect trehalase



Ryanodine
Effector of calcium release channel



Allosamidin
Potent inhibitor of chitinases of various origin

Fig. 2. Structures of putative antiparasitic lead structures. Compounds are shown for which a primary target site is likely to be of interest for the discovery of new antiparasitic drugs.

spin-offs for anti-nematode treatment were even more limited and only a few acetylcholinesterase inhibitors, such as diazinon or coumaphos, were used in both fields. Classes of nematicidal compounds with a wide spectrum of activity, such as imidazothiazoles or benzimidazoles, although quite successful against animal parasites, have never achieved any significance in nematicide-mediated crop protection. This indicates that physicochemical parameters like soil adsorption, degradation, tissue distribution etc., are specific key factors limiting broad applicability, besides activity at the target site.

During the last decade the cost of development of a product for animal-health use (≧US $35 million for development; registration costs not included) in livestock and companion animals has increased significantly. The limited market size, together with concern about resistance and critical side effects of arthropodicidal compounds which affect classical targets (acetylcholinesterase; sodium channel; GABA gated chloride channel), have led to the introduction of only a few new products displaying a new mode of action, which is also reflected by the drastic decrease in patent applications.[3,4]

TABLE 2
Newer Arthropodicidal and Nematocidal Compounds Discovered from Natural Sources[a]

Compound	*Biological activity*[b]	*Producing organism*	*Method of screening*	*Mode of action*	*Reference*
AB-3217-A	I	*Streptomyces platensis*	Tetranychus assay	Unknown	148
Angulatin	I	*Celastrus angulatus*	Heliothis and other assays	Unknown, antifeedant, insecticidal	149
Allosamidin	I	*Streptomyces*, sp. No. 1713	Chitinase screening	Chitinase inhibitor	150
Altemicidin	I, C	*Streptomyces sioyaensi*	Brine shrimp, Tetranychus assay	Unknown	151
Annonin	I, C, N	*Annona squamosa*	Brine shrimp, Caenorhabditis elegans	Inhibitor of site I respiratory chain	152
Bagougeramine A	M	*Bacillus circulans*	Tetranychus assay	Unknown	153
Cochlioquinone A	N	*Helminthosporium sativum*	Displacement [^{3}H] ivermectin	Glu-gated chloride channel	154
Dioxapyrrolomycin	N, I, M	*Streptomyces* sp. MG 796AF7	Insecticidal bioassay	Uncoupler of oxidative phosphorylation	16
Iietacins	N	*Streptomyces* sp. KP-197	Bursaphelenchus assay	Unknown	155
Leucanicidin	I	*Streptomyces halstedii*	Leucania (armyworm) assay	Unknown	156
Okaramine B	I	*Penicillium simplicissimum*	Silkworm assay	Unknown	157
Paraherquamide	N	*Penicillium paraherquei*	Caenorhabditis assay	ACh and/or phenothiazine binding site	17,87,88
PF1022 (Depsipeptide)	N	*Agonomycetales*	Heterakis, Ascaridia assay	Chloride channel?	18,65,66
Spinosyn	I	*Saccharopolyspora spinosa*	Insect bioassays	Unknown	158
Trehalostatin	I	*Amycolatopsis trehalostatica*	Trehalase Screening	Trehalase Inhibitor	159
Validoxylamine A	I	*Streptomyces hygroscopicus*	Trehalase Screening, Spodoptera assay	Trehalase Inhibitor	160
L155, 175	Ct	*Streptomyces hygroscopicus*	Hymenolepis assay in rats	Unknown	161

[a] Omitting newer avermectins or milbemycins.
[b] I, insecticidal; N, nematocidal; M, miticidal; C, active against crustaceans; Ct, active against cestodes.

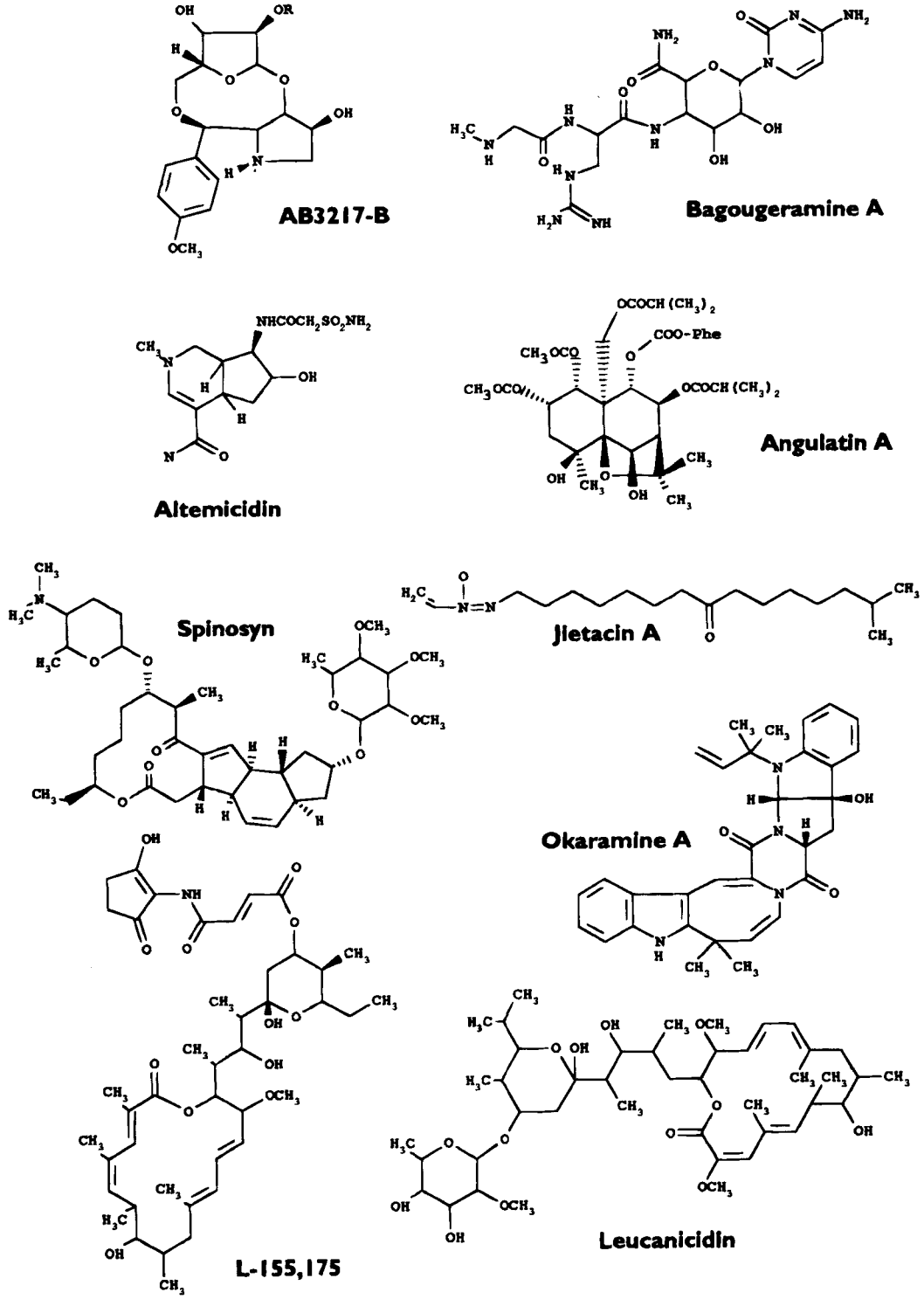


Fig. 3. Newer natural products with parasiticidal activity; see Table 2.

In the past, most known pesticides were discovered *via* empirical and analogue synthesis in combination with conventional screening and optimization using structure–activity relationships.[5] In recent years, a few new products with an interesting broad biological activity have been identified, particularly those involving compounds of natural origin such as the avermectins[6] and new insecticides like the chloronicotinyl[7] compounds. So far, these and other interesting parasiticides from various origins, have been sparingly exploited as leads to establishing new screening systems. With the increasing knowledge of arthropod and nematode physiology, particularly with regard to $GABA_A$ receptors,[8] muscarinic and nicotinic acetylcholine recep-

tors,[9,10] cuticle degradation,[11] ecdysteroid receptors,[12] the calcium release channel[13] and cloning of glutamate-gated chloride channels,[14] to name only a few of the relevant target sites, the time for directed biorational search for new leads may now have arrived. These screening tools, together with classical biological random screening, will identify sites in parasites that are amenable to selective manipulation by therapeutics. When combined with methods to combat parasites immunologically, the two approaches will be long-term 'running mates' in application schemes comparable to integrated pest management in crop protection. It appears that the trend in most research organizations—from an overview of patent literature and informal discussions with many colleagues, rather than on specific statistics or references—is towards developing a balance of the four strategies discussed below in this review.

2 NEW CHEMICAL STRUCTURES AND NATURAL PRODUCTS

Considering the current state of synthetic methodology, the range of commercially applicable chemistry is constantly expanding and there seems to be no direct correlation between structural complexity and biological activity. Since more high-capacity screening systems are now available, many additional 'simple' molecules with high biological activity can be expected to be found. Selected recent examples are the ecdysteroid agonist RH 5849 (tebufenozide)[12] and the broadly active acaricide, fenazaquin, which acts as an inhibitor of site I of the respiratory chain.[15] We have probably only scratched the surface in the area of surveying naturally occurring substances for direct use as therapeutics or lead structures, recent examples of which are the extremely successful avermectins, the dioxapyrrolomycins as lead structures for AC303630,[16] the anthelmintic paraherquamides,[17] and the nematicidal cyclodepsipeptide PF1022A (Figs 1, 2, Plate 1).[18]

Microbial metabolites and biologically active substances from plants are gaining increasing attention as potential parasiticides, particularly after the commercially very successful introduction of avermectins and milbemycins.[19] In drug-research-oriented institutes and companies there have been various attempts to identify new microbial metabolites with high biological activity or interesting sites of action which are different from those reported in recent years.[20] These efforts have led to a significant increase in the discovery of non-antibiotics[6] displaying antiparasitic activities (Table 2), and were particularly driven by new screening systems and by expansion of the microbial genera tested, which involved basidiomycetes, filamentous fungi etc., as well as actinomycetes. So far, even when dealing with probably less than 10% of the entire microbial population of the ecosystem,[6] a variety of different, highly active parasiticides have been detected using biological as well as biochemical screening systems. This suggests convincingly that it should be possible to identify new bioactive metabolites which fully match the following criteria for new parasiticides. (1) The problems of breaking resistance incurred with market products; (2) high and broad-spectrum activity against nematodes and/or arthropods; (3) harmless to the environment, the user and the consumer and (4) cost-effective and compatible with other products used.

As shown in Table 2 and Figs 2 and 3, various new natural parasiticidal drugs were discovered in recent years using different screening approaches. Bioassays are still the predominant tool to identify new chemical entities, but biochemical test systems are gaining increasing relevance in primary screening approaches.

One system which has not so far been exploited in commercial terms, but which is still a very relevant target site, is the calcium release system of excitation–contraction coupling.[21] A variety of compounds affecting calcium channels of the N-, T- or L-type have been identified in vertebrate preparations on the basis of their electrophysiological or pharmacological effects.[22–24] In arthropods, binding sites for phenylalkylamines, 1,4-dihydropyridines and ryanodine have so far only been identified in different insect tissue preparations.[13,25,26] Comprehensive studies within one insect species concerning the contribution of different calcium channel types to the excitation–contraction coupling are still incomplete. Biological investigation of ryanodine (Table 3) clearly indicates the potential of the calcium release channel and associated proteins as targets for insect control.[27] Ryanodine itself, however, seems to be a very potent, but nonselective, probe for

TABLE 3
Biological Activity of Ryanodine and 9,21-Didehydroryanodine against Different Arthropods of Importance for Animal Health and Crop Protection

Compound	LC_{50}^{a}							
	Boophilus microplus	*Locusta migratoria*	*Aedes aegypti*	*Phaedon cochleariae*	*Plutella maculipennis*	*Spodoptera frugiperda*	*Heliothis armigera*	*Tetranychus urticae*
Ryanodine	30–50	0·2–0·4	0·1–0·3	0·01–0·03	0·03–0·08	0·5–0·8	4–10	100–400
9,21-Didehydroryanodine	10–20	0·3–0·7	0·5–1·0	0·03–0·05	0·03–0·08	0·5–0·8	0·8–4	200–600

[a] In terms of $\mu g\ g^{-1}$ body weight for *B. microplus* and *L. migratoria* and of $\mu g\ ml^{-1}$ for the other targets.

calcium release channels which is toxic to invertebrates and vertebrates.[25] Since the structurally closely related ryanodol is much less toxic than ryanodine to mice,[25,28] it can be assumed that specific binding sites exist in insects, which should allow the exploitation of calcium channels involved in insect excitation–contraction coupling by high-capacity screening systems for new compounds, whether structurally related to ryanodine or not. Striking structural differences exist also on the molecular level. The cytoplasmic domains of the *Drosophila* spp. α_1 subunit of the calcium channel are much longer than in mammalian skeletal muscle and there is also no similarity in this region between the insect and the vertebrate sequences.[21] In addition, a comparison of cytoplasmic, transmembrane and extracellular domains of *Drosophila* spp. and rabbit skeletal muscle α_1 subunit has revealed numerous extracellular domains which were only weakly conserved.[21] So far, the functional significance of these regions is not clear. One can speculate, however, that synthetic drugs, which most often display highly liphophilic properties, can act as membrane-permeating agents and function as insect-specific toxins.

In this context, the recent discovery of bastadin, an enhancer of ryanodin binding from a sponge (*Ianthella* spp.)[29] suggests that modulators of calcium release channels, not structurally related to ryanodine, might in addition be a so-far-unexploited target for new anti-parasitic drugs.

3 BIO- AND 'SOFT'-TECHNOLOGIES

The field of animal health biotechnology particularly involves stimulation of the host immune system to combat invading parasites. A typical example is the cattle-tick vaccine against *Boophilus microplus* Can. launched recently in Australia. After an initial primer immunization and two to three booster injections, few additional acaricide treatments,[30] depending on the tick pressure, are necessary to control this parasite. By this approach the development of acaricide resistance should be retarded and concerns among the consumers about residues in meat and milk can be reduced. Awareness for protection of the environment, and hence the demand for more selective products, will enhance prospects of 'soft' technologies. This means predominantly the application of microbial pesticides such as *Bacillus thuringiensis* Berliner, insect pathogenic fungi (e.g. *Beauveria bassiana* Vuill., *Metarhizium anisopliae* (Metsch) Sorokin, *Verticillium lecanii* (Zimm.) Niegas, insect viruses (nuclear polyhedrosis virus) and insect parasitic nematodes (e.g. *Steinernema* spp., *Heterorhabditis* spp.).[31] The inherent specificity of most, but not all, biological control techniques will challenge the integration of biotechnology beyond the prevailing view that they are independent, competing solutions of agrotechnical problems. Together with other forms of control, particularly the combatting of pests and parasites with repellents (Fig. 4),[32] and soaps, detergents and oils,[33] these types of treatment will achieve a substantial market share.

Host-seeking behaviour of blood-feeding arthropods essentially involves orientation to a host from a distance. The use of semiochemicals to manipulate insect behaviour has proved to be one of the important components of integrated pest management (IPM) programmes and personal protection measures. In IPM programmes, sex pheromones[34] in particular have been used for monitoring, behavioural disruption, mass trapping and insecticidal lures against a variety of insects, including orchard, cotton, or forestry pests[35,36] and cockroaches.[37,38] Among blood-sucking arthropods affecting humans, repellents play the predominant role because they can be used very easily. *N,N*-diethyl-*m*-toluamide (DEET) is most frequently applied in this area.[39] The use of repellents is advantageous in view of the fact that legislation and regulatory authorities are becoming more and more restrictive towards the use of conventional pesticides. A number of countries and governments have set themselves ambitious targets for reducing insecticide usage by specific time deadlines and this trend will probably continue as political pressure increases.

Various products from natural sources, as well as synthetic compounds, have been used as repellents, sometimes exploiting the repellency side effects of conventional neuroactive compounds. This involves e.g. cinerins, pyrethrins and jasmolins (*Chrysanthemum cinerariaefolium* Vis.), citronellol, indalone, acorus and garlic oil, as well as DEET, *N,N*-diethylphenylacetamide (DEPA) and dimethylphthalate (DMP). The efficacy of arthropod repellents is not only determined by the proposed receptor interaction, but also by such other factors as physicochemical properties, penetration

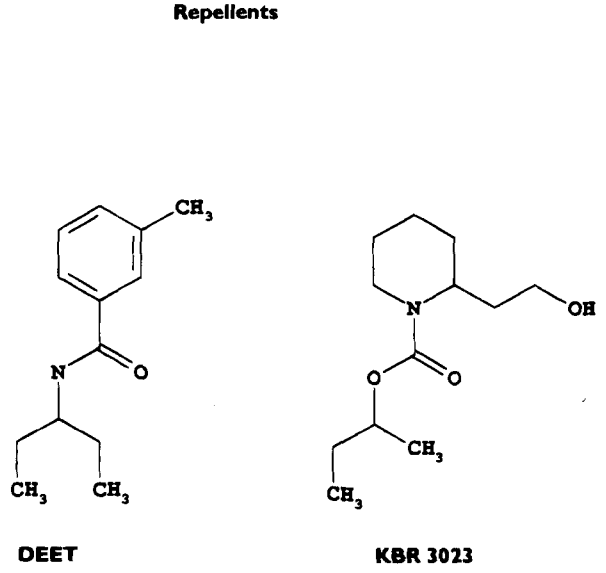


Fig. 4. Structures of DEET and a new repellent, KBR 3023.

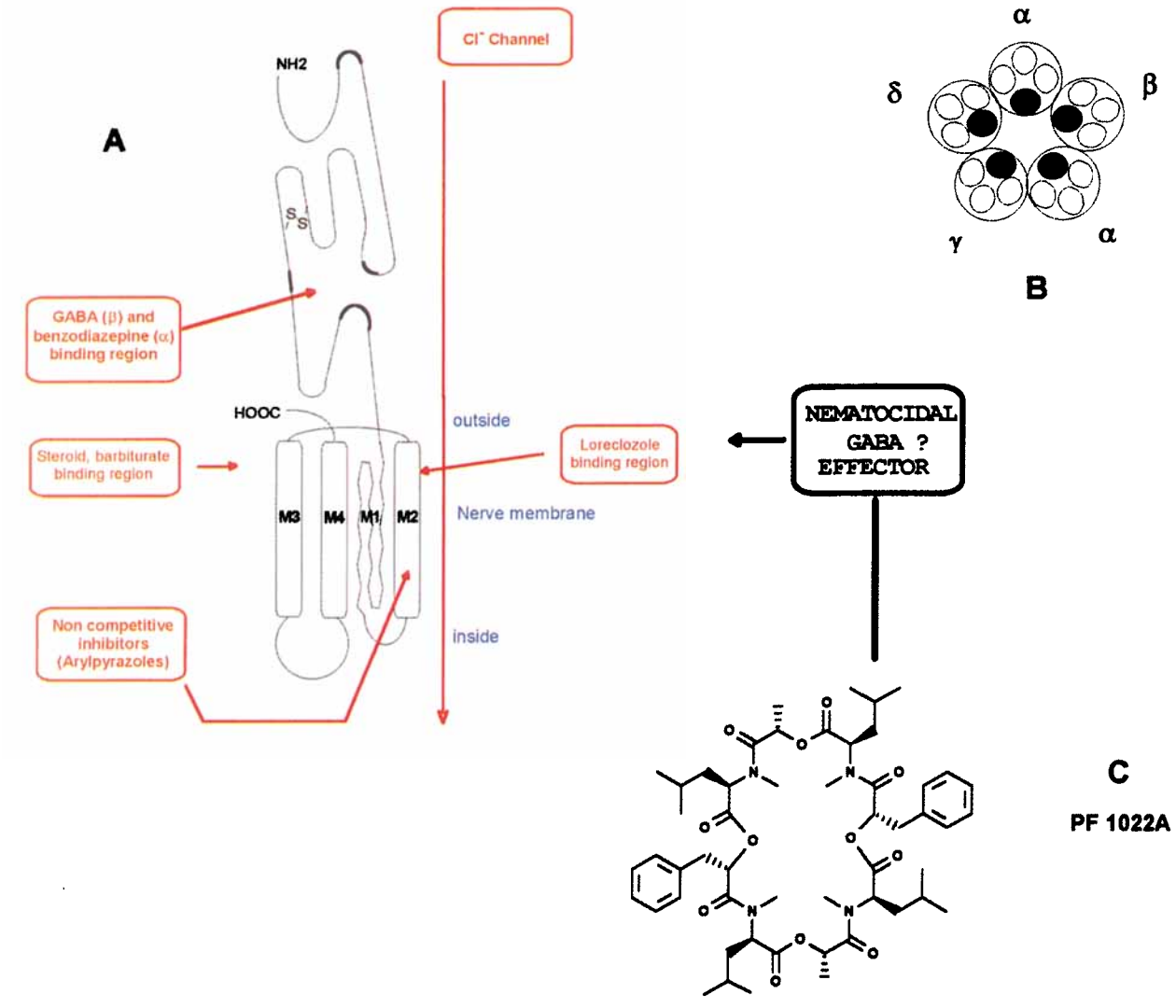


Plate 1. Schematic representation of the GABA-activated chloride channel with binding sites for different effectors. In **A** the GABAR α-subunit of mammalian brain is schematically shown, with regions of effector binding indicated. Those sequences considered to be important for GABA and benzodiazepine binding are indicated by bold lines. The mammalian GABAR consists of five subunits (**B**): α, β, γ, δ, and ρ for which different isoforms also exist: $\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, $\rho_{1\text{-}2}$.[53] The new anthelmintic depsipeptide PF1022A (**C**) displayed some activities on the GABAergic system of nematodes. A clearly defined binding site has not been identified so far, but if the GABAR is a primary target site of this drug, binding sites at the membrane level are likely, since it displays extremely lipophilic properties.

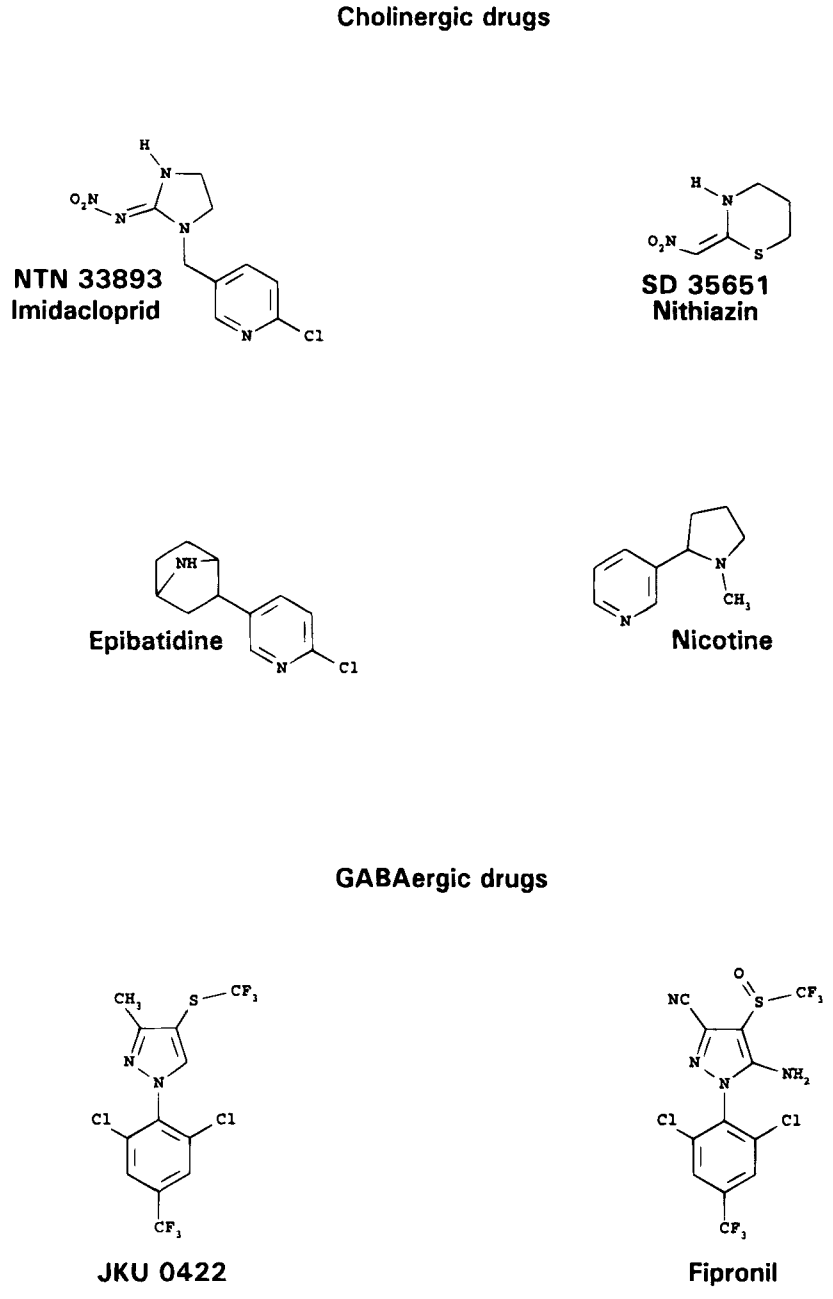


Fig. 5. Structures of cholinergic and GABAergic drugs.

rate, perspiration, metabolism, population density, environmental factors and type of formulation.[40] In addition, different organisms differ widely in their response to repellents, examples being the limited value of DEET in protection against ticks[41,41] and *Anopheles albimanus* Wied..[43] The search for new compounds with a broader spectrum of activity therefore remained solely empirical for years,[39] since repellency could well be the result of superficial reactions on the antennae which cause higher nervous centres of insects to elicit avoidance reactions. Even if the mode of action of repellents is still an open question, computer-aided design, oriented at a supposed DEET-binding protein interaction, was used to guide chemical synthesis of new repellent structures. An acylated piperidine derivative (KBR 3023) was discovered by this drug design approach. The compound was clearly superior in repellent activity when compared with DEET and DMP against the tick *Rhipicephalus sanguineus* Latr., the mosquitoes *A. albimanus* and *Culex quinquefasciatus* Say and the stable fly *Stomoxys calcitrans* L. In general the compound displayed a wider range of activity than other repellents and was also superior regarding its safety and low potential to cause skin irritations and softening of different plastic materials.[44] Whereas repellents have been mainly used to protect humans and animals, e.g. horses, against bloodsucking arthropods, recently the possibility of developing semiochemical baits against the

important sheep blowflies *Lucilia sericata* Meig. and *Lucilia cuprina* Wied. has been reinvestigated. A higher proportion of gravid females were attracted by sulphur-rich volatiles originating from bacterial decomposition products when compared with non-gravid females or males.[45] This approach gives hope that odour baits might also be used successfully in the future against important sheep parasites. In this context the resistance and environmental impact of the widespread use of organophosphate insecticides applied for blowfly strike has renewed interest in alternative control techniques.

4 NEW IDEAS IN KNOWN AREAS

This approach depends on already known compounds as starting points, but creative chemists may add new value to existing lead structures. The initial 'hit' is seldom the optimum in any area. Innumerable examples of this may be cited, but a few are particularly notable. In 1984 the unexpected insecticidal activity of 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazoles was discovered[46] and later significantly improved by the introduction of a cyano-substituent in the 4-position of the heterocycle.[47] Originally these compounds were synthesized as herbicides, but it was shown that JKU 0422 and fipronil acted similarly to dieldrin or lindane at the GABA-gated chloride channel.[48] Another successful example was the synthesis of arthropod-specific chloronicotinyl-nitroguanidines (e.g. imidacloprid, SD 35651), which were shown to be agonists of an insect nicotinic acetylcholine receptor type which binds α-bungarotoxin with low affinity (Fig. 5).[7,49]

5 BIORATIONAL DESIGN OF THERAPEUTICS

The biochemically guided strategy within agrochemical research has yet to result in commercial success. Nevertheless optimism is justified, since this strategy can be supported easily by cooperation with university biochemistry and biotechnology research groups. Specific new modes of action can be selected 'intentionally'. Poor correlation between *in-vitro* enzyme- or receptor-inhibition and whole-organism activity can help to improve our knowledge about metabolism, tissue distribution and xenobiotic penetration.

The selection of target sites depends mainly on the intuition of research groups as to which candidates fit best with the demands of the market. Among others, existing, successful market products affecting known target sites, as well as a reliable supply of target organisms/enzyme systems for testing purposes, provide one useful framework for the selection. Examples are given in Table 4. Also important are target sites for which a high biological activity has been demonstrated convincingly by lead compounds and these are useful for guided synthesis. This approach requires the establishment of a suitable screening assay. Natural products may also provide valuable leads for antiparasitic agents, as shown in Table 4.

5.1 Target fields of arthropodicidal and nematicidal drugs

With regard to the phylogenetic differences between parasites and their vertebrate hosts, two basic target fields seem to be particularly suitable for the design of arthropodicidal and nematocidal drugs.

5.1.1 Neurotransmission

Despite the fact that arthropods, nematodes and vertebrates basically use the same neurotransmitters, various differences have been identified at the physiological and molecular level in the target sites concerned. The increasing fundamental knowledge, particularly on ion channels, gives hope that these targets can still be

TABLE 4
Interesting Target Sites for New Parasiticides Active against Arthropods and/or Nematodes

Target	*Effector compound examples*	*Function regulated*	*Reference*
Chloride channel (GABA, glutamate-gated)	Avermectins, Cochlioquinone, A, fipronil, milbemycins, PF1022 (depsipeptides),	Neurotransmission	48,65,66,154,162
Nicotinic acetylcholine receptor	Chloronicotinyls, epibatidine	Neurotransmission	7,49,75
Muscarinic acetylcholine receptor	Oxotremorine, QNB	Signalling mechanisms, development	9,93
Respiration/uncoupling	AC303630, annonin, dioxapyrrolomycin, fenazaquin, fenproximat, pyridaben	Energy supply	16,152
Calcium-release channel	Ryanodine	Excitation contraction coupling	13,163
Chitin-synthase/-synthesis	Benzoylphenylureas, polyoxins nikkomycins	Development, moulting	115,128
Repellent receptors	KBR 3023, *m*-diethyltoluamide	Behaviour	44
DOPA-decarboxylase	Carbidopa	Sclerotization, development	131

exploited for drugs revealing a sufficient therapeutic index and a resistance-breaking activity. The advantage of a quick onset of a broad biological activity of such compounds faces the disadvantage of being generally less species-specific. The study of neurotransmitter receptor molecules has been stimulated by the growing realization that their basic understanding represents an essential tool in any attempts to exploit such target molecules for the rational design of new classes of parasiticides. With regard to ion channels, ligand-gated chloride channels and the nicotinic acetylcholine receptor seem to be particularly useful targets for antiparasitic compounds. Therefore they have been taken as examples to illustrate the previously mentioned underlying ideas.

5.1.2 Interference with growth and/or endocrine function
In contrast to neurotransmission, unique target sites have been identified in parasites which do not exist in vertebrates. Consequently, the 'toxicity gap' should be large enough to identify and design highly parasite-selective drugs. Since the relatively slow onset of activity is a clear disadvantage of at least the growth regulators, and restricts their broad applicability, interference with hormonal regulation, cuticle formation and peptide hormone processing are discussed to exemplify this approach.

5.2 GABA receptors

Nematodes, as well as insects, use mainly the same neurotransmitters as vertebrates, but their receptors show distinct pharmacological differences.[50,51] In the case of antiparasitic drug research, these species-specific differences may be exploited by developing selective insecticides, acaricides or nematicides. 4-Aminobutyric acid (GABA) is a major inhibitory transmitter at the neuromuscular junction not only in nematodes[52] but also in insects.[51] In vertebrates, two subtypes have been identified: $GABA_A$ receptors which contain a ligand-gated Cl^- channel as well as modulatory sites for various drugs; and $GABA_B$ receptors, G-proteins, which mediate GABA effects on Ca^{2+} and K^+ channels.[53] So far, with the exception of $GABA_B$ receptors on *Periplaneta* spp. motoneurone D_f,[54] most receptors identified in nematodes and insects resemble the $GABA_A$ subtype (Plate 1), but they are clearly distinct from their vertebrate counterparts.[55,56]

The agonist profiles of several nematode or insect $GABA_A$ receptors show similarities with the corresponding vertebrate receptors. Muscimol is equally, or more, active than GABA, and isoguvacine also shows some potency.[51,57] In general their antagonistic profile is quite different from vertebrates and varies also between nematodes and insects. Whereas, in insects, bicuculline-insensitive (cockroach and locust CNS)[56] and sensitive (tobacco horn worm)[57] receptors have been identified, convulsant antagonists such as picrotoxin, bicuculline and TBPS (*tert*-butyl-bicyclo[2.2.2] phosphorothionate) all failed to block GABA receptors of *Ascaris* spp. muscle.[58]

A *Drosophila* spp. GABA-gated chloride channel subunit cDNA has recently been cloned using genetic mapping of a mutation, underlying insecticide resistance against dieldrin (Rdl).[8] By means of electrophysiological investigations of *Drosophila* spp. Rdl homo-oligomers expressed in *Xenopus* spp. oocytes,[59] as well as nerve preparations of dieldrin-resistant field strains[48] of *Drosophila* and *Blattella germanica* L., the GABAergic effects of a new insecticide and acaricide chemical class — the arylpyrazoles — was confirmed. Two phenylpyrazoles, fipronil and JKU 0422 (Fig. 5), were shown in these studies to act as convulsant antagonists at the TBPS/cyclodiene binding site. Since cyclodiene resistance extended to JKU 0422 for a German cockroach strain (LPP), the use of these new chloride channel antagonists as parasiticides should be managed carefully in order to prevent rapid development of resistance in the field. By contrast, for certain insects important as crop pests, it was demonstrated that fipronil was active against both susceptible and dieldrin-resistant strains.[60] Among the compounds successfully applied in the market, the channel blockers are of particular interest (Table 5). Presumably they bind to the putative *trans* membrane region 2 (TM2) of the receptor, since resistance against picrotoxin and dieldrin in a *Drosophila* mutant (Rdl) was conferred by a single point mutation in this region of Ala302 → Ser.[59] A ranking order of potency of *Drosophila melanogaster* L. wild-type GABA receptor homo oligomers expressed in *Xenopus* oocytes revealed, for agonists: GABA ≈ muscimol ≈ TACA (*trans*-4-aminocrotonic acid) > CACA (*cis*-4-aminocrotonic acid) > glycine, and for channel blockers: picrotoxin > EBOB (4-*n*-propyl-4′-ethylnylbicycloorthobenzoate) > fipronil > TBPS.[59] Discrepancies between in-vitro studies (receptor binding assays, electrophysiological investigations) are due most often to the sometimes drastically different pharmacokinetic behaviour in the parasite. In addition, for more hydrophilic compounds, the cuticle barrier often prevents diffusion to the target site.[61] The good activity of channel blockers against ticks suggests that at least this binding site resembles the insect GABAR, even if nearly nothing is known at the molecular level of GABARs of ticks. In contrast to the results of arthropodicidal activity in nematodes, all known non-competitive antagonists of mammalian or insect GABARs were either inactive or only very weak antagonists.[62]

Nematode chloride channels have also been shown to be a suitable target for antiparasitic activity, as was demonstrated for avermectin-sensitive glutamate-gated chloride channels from *Caenorhabditis elegans* Maupas.[14] With regard to antagonist specificity, nema-

TABLE 5
Biological Activity of GABAergic Drugs

Effectors		*Insect bioassay*[a]	*Nematode bioassay*[a]	*Tick bioassay*[b]
Agonists				
	GABA	–	–	–
	Muscimol	+(+)	+	–
	Isoguvacine	(+)	–	–
Antagonists				
	Bicucullin	(+)	–	–
Benzodiazepine binding site				
	Flunitrazepam	–	–	–
Channel blockers				
	JKU 0422; Fipronil	+++	+++	+++
	Picrotoxin	+	–	++
	TBPS	(+)	–	(+)
	α-Endosulfan	+++	+	+(+)
	γ-HCH	+++	+	+++
Others[c]				
	Avermectin Blα	+++	+++	+++
	β-Cyfluthrin	+++	–	+++
	PF1022A	–	+	–

[a] Insecticidal and nematocidal activity were determined using an *Aedes aegypti* larval assay (50 L_3 50 ml^{-1} water) and a *Caenorhabditis elegans* test using a suspension culture. Culture medium (50 µl) containing the compound under investigation was added and the test was investigated at two times. The results were rated according to the following scheme: – no effect; + symptoms within 24 h (reduction of excitability, paralysis, hyperexcitability); ++ severe symptoms within 8 or 24 h, +++ mortality within 24 h, ( ) poor activity.

[b] Acaricidal activity was compared qualitatively according to the ranking: – 0%; +≦40%; ++ 40–70%; +++ 70–100% mortality. The compound in DMSO (20 µg μl^{-1}) was injected (1 µl) into adult, fully engorged females of *B. microplus* (300 mg weight per tick).

[c] Compounds with GABAergic side effects or presumed GABAergic activity.

tode GABA receptors seem to be significantly different from those in arthropods. It would seem that the *Ascaris* GABA receptor recognizes few of the compounds that have been used to characterize the pharmacology of insect or mammalian systems.[62] Interestingly, an inhibitory amino acid receptor unit has recently been cloned from *Haemonchus contortus* Rud.[63] which responds to glycine but not to GABA. In this context, the Rdl gene from *Drosophila* shows somewhat higher homologies with glycine receptors than with GABA receptors.[64] The screening of herbicidal phenylpyrazoles in insecticidal test systems led to a new class of promising arthropodicidal drugs displaying GABAergic effects. Since the importance of this neurotransmitter was also demonstrated for nematodes[50] it is hoped, that new drugs can be found, which exert their nematocidal effects through this target site. The investigation of new fermentation extracts is one approach to achieve comparable success.

The very recent discovery of a completely new class of anthelmintic C_2-symmetric cyclodepsipeptides (e.g. PF1022A, consisting of four *N*-methyl-L-leucines, two β-phenyl-D-lactic acids and two D-lactic acids),[18] which seem to exert their activity at least partially through GABAergic effects,[65,66] demonstrates this impressively. These cyclodepsipeptides affect a nematocidal, sterero-selective target site. PF1022A, but not its biologically inactive antipode (PF1022-001, consisting of four *N*-methyl-D-leucines, two β-phenyl-L-lactic acids and two L-lactic acids) produced a time-dependent flaccid paralysis of somatic muscles of *Ascaris suum* Goeze. The effect was associated with a hyperpolarized membrane potential of a few mV.[67] In addition, PF1022A after long-term incubation (≧5 h), suppressed irreversibly the amplitude and the frequency of spike events during the autorhythmic depolarization of *A. suum* nerve-muscle preparations. Similar, but reversible effects can be achieved after application of the neurotransmitter GABA. These data, together with the demonstration of stereoselective specific binding sites for PF1022A in *A. suum* muscle preparations[68] and in *in-vitro* effects of PF1022A on GABA receptors of *A. suum*[69] indicate that chloride channels, perhaps modulated by GABA, might play an important role in the mode of action of this new class of compounds (Plate 1). This is consistent with effects observed for another depsipeptide, the insecticide, bassianolide, which was shown to inhibit TBPS binding with an IC_{50} of 7 µM.[70] Similar to other cyclodepsipeptides such as valinomycin and enniatin A, PF1022A acts as an ionophore in lipid bilayers. So far, it is not clear as to what extent the ionophoric properties of PF1022A contribute to the primary mode of

action of the drug. Since a series of related depsipeptides, e.g. the antipode PF1022-001, displayed carrier properties but no nematocidal activity, it is concluded that ion-carrier function is of minor importance for the paralyzing effects on nematodes.[67]

Since depsipeptides consist of a regular, repeating pattern of distinct hydroxy-carboxylic and amino acid units, the open-chain products in particular offer the chance to establish compound libraries *via* a combinatorial chemistry approach. The number of compounds produced depends on the chain-length and how frequently each amino acid and/or hydroxy-carboxylic acid is allowed to appear. For example, a full permutation of only eight units would lead to more than 25×10^9 different chemical entities. This would by far exceed the capacity of any biological in-vivo screening system. Assuming that the relevant target sites have been identified and cloned, characterization of the distinctly bound depsipeptides out of the enormous pool of molecules with low affinity might be achieved after some kind of competition assay. The potency of parasiticides influencing the GABAergic system has been increased to the point where they approach the effectiveness of most pyrethroids and of substances acting on the cholinergic system. Selective toxicity might not be achieved easily, but it has been shown that the binding sites in nematodes and insects differ sufficiently from those in vertebrates to suggest that species-specific inhibitors can be identified.

5.3 Cholinergic receptors

5.3.1 Nicotinic-cholinergic receptors

Cholinergic synapses play a major role in transmission in arthropods and nematodes.[10] Recently, imidacloprid (IMI) was launched as a novel insecticide displaying a new mode of action.[7] Chloronicotinyl insecticides of the IMI type act at the nicotinic acetylcholine receptor (nAChR, Plate 2), as was demonstrated for a number of insects by biochemical and electrophysiological techniques.[71–73] To some extent IMI and its analogues displace [^{3}H]α-bungarotoxin from its binding sites, but they are almost 3000-fold more potent as inhibitors of [^{3}H]IMI binding.[61] The weak displacement of [I^{125}]α-BGT by SD-35651 at nACRs from male *Periplaneta americana* L. brain tissue[74] indicates that chloronicotinyls affect a distinct new nAChR binding site in insects. It was surprising that the chloropyridyl moiety of the nicotinyls, which is essential for high activity in this class of compound, was already used by 'Mother Nature' a very long time ago. Epibatidine (Fig. 5), an alkaloid from frog skin, contains the same chloropyridyl moiety and was shown to be a potent nicotinic agonist in rats and mice.[75] Epibatidine is only a weak competitor for imidacloprid binding (Table 6), which is another indication of differences between invertebrate and vertebrate nAChRs, but it exemplifies the possibility of using structural moieties present in nicotinyls as 'toxophoric components' in order to improve the species-specific activity of defined compounds. nAChRs from mammalian sources are much less, or not at all, sensitive to the agonistic action of insecticidal chloronicotinyls or nitromethylenes. This is supported by the fact that no neurotoxic effects were observed in toxicity studies on rats. Various subtypes of nAChRs exhibit significant differences in sensitivity towards nicotinyls which allows selective toxic effects to be exerted by different ligands.[76] In this context, compounds like imidacloprid may become important tools to characterize a new insect-selective molecular target: a subtype of nAChR, which is essential for insect neurofunction but which is different in pharmacology and tissue distribution from all mammalian nAChRs investigated so far.[77]

In addition to the recognition of an insect-specific nAChR binding site[78] by imidacloprid and its analogues physicochemical properties of chloronicotinyls may not only account for the low vertebrate toxicity but also for arthropod species-selective effects. For example, imidacloprid is active against flies, fleas, cockroaches and mosquitoes if applied effectively. It is believed that, in contrast to nicotine, having entered the body, the compound is not ionized and is easily transferred into the central nervous system, where nAChRs are predominantly located in insects.[76] This would also explain the low toxicity of imidacloprid in vertebrates compared to nicotine.[76]

To a lesser extent, chloronicotinyls are also active against ticks (*B. microplus*) and nematodes (*Haemonchus contortus* Rud.) (Table 6). In *A. suum* or *C. elegans*, levamisole, pyrantel and amidantel have been shown to be highly active agonists at AChRs,[79,80] but levamisole and pyrantel were only weakly active (100 times less than carbachol) in depolarization of motoneurone D_f of *P. americana* coxal depressor. Since the antagonist mecamylamine abolished these weak responses, it was concluded that the drugs were acting at the same site.[81] The nitromethylene insecticide NMTHT had no effect on *A. suum*, while ACh elicited depolarization and an increase in input conductance of muscle cells at up to 1 mM, indicating the differences between AChRs in insects and nematodes. Similar results were obtained when [^{3}H]IMI was used to characterize nAChRs in flea (*Ctenocephalides felis* Bche.) and *A. suum* membranes. IMI displaced [^{3}H]IMI with an IC_{50} value of 10 nM in fleas, whereas no binding could be detected in *A. suum* muscle membranes (Tietjen, K., Londershausen, M. & Turberg, A., 1995, unpublished). In accordance with the weak effects of pyrantel on *P. americana* motoneurone D_f, this compound revealed an IC_{50} value of only 0·3 μM in the flea preparation (Tietjen, K., Londershausen, M. & Turberg, A., 1995, unpublished). In some respects, however, nematode nAChR resembles the nAChRs from insect nervous systems. For example, the antagonist *N*-methyllycaconitine blocked *A. summ*

TABLE 6
Potencies of Cholinergic Drugs

Effectors	*nAChR-agonist binding assay*[a]	*Insect bioassay*[b]	*Tick bioassay*[b]	*Nematode bioassay*[b]
Nicotinic agonists				
Imidacloprid	1·0	+++	–[c]	–[d]
(+)-Anatoxin A	50	+	–	(+)
Epibatidine	1000	++	–	(+)
Nicotine	2512	+(+)	–	(+)
Cytisine	3162	+	–	+
Acetylcholine	>20000	–	–	(+)
DMPP	>20000	–	–	+(+)
Levamisole	>20000	–	–	+++
Pyrantel	>20000	+	(+)	+++
Nicotinic antagonists				
N-methyllycaconitine	1.6	++	–	–
Dihydro-β-erythroidine	10	+	–	(+)
α-Bungarotoxin	1585	+	–	–
d-Tubocurarine	~20000	+(+)	+	–
Decamethonium	>20000	–	–	–
Hexamethonium	>20000	–	++	–
Mecamylamine	>20000	–	–	–
Muscarinic agonists				
Bethanechol	n.t.[e]	–	–	–
Carbachol	n.t.	+	–	–
Muscarine	>20000	+	(+)	–
Oxotremorine	>20000	–	+++	–
Muscarinic antagonists				
Atropine	>20000	–	–	+
4-DAMP	n.t.	(+)	++	–
Methoctramine	n.t.	(+)	–	–
Pirenzepine	≦20000	–	(+)	–
Quinuclidinyl benzilate	~20000	–	–	+[f]
Scopolamine	>20000	(+)	–	–

[a] In the table various cholinergic agonists and antagonists are compared with regard to their ability to displace radiolabelled imidacloprid (binding assays were performed[61] at 0·36 nM [^{3}H]IMI using a *Musca domestica* membrane preparation (Tietjen, K., Bayer AG, 1995, pers.comm.). Relative potencies are expressed as the relative proportion of [^{3}H]IMI displacement by IMI (IC_{50} 0·8 nM, which was set as 1).

[b] Biological assays were performed and results are expressed as explained in Table 5.

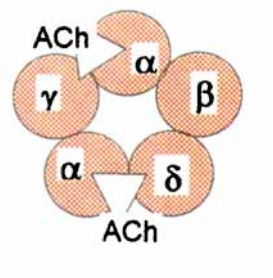
[c] A tick packet assay was performed with 20 *B. microplus* larvae which were kept for five days between two paper sheets impregnated with 1 ml of a solution containing 1 mg ml^{-1} of the compound under investigation. The compound killed 100% of the tick larvae.

[d] *Haemonchus contortus* nematodes were tested by applying 10 mg kg^{-1} body weight to sheep which were fully infested with an *H. contortus* population. The compound displayed 100% activity against this parasite.

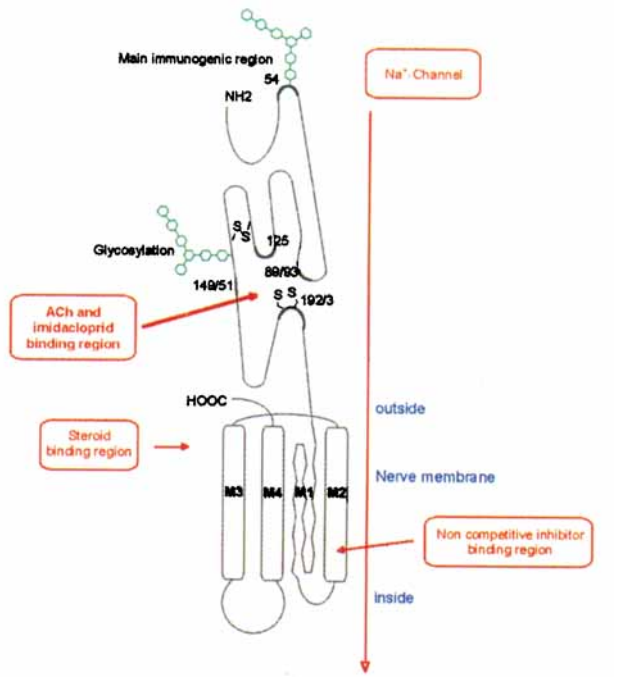
[e] n.t.: not tested;

[f] Binding in nM range has been demonstrated.[93]

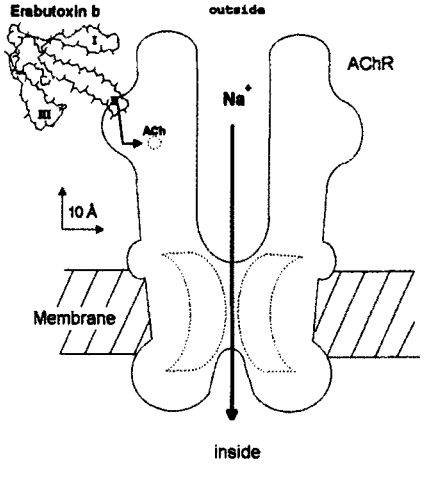
muscle nAChRs and neuronal nAChRs from cockroaches very effectively,[82] whereas hexamethonium, a powerful vertebrate nAChR antagonist, was nearly ineffective on *Ascaris* muscle[83] and *Manduca sexta* L. nAChRs.[84] By contrast, 1,1-dimethyl-4-phenylpiperazinium (DMPP), a highly active agonist of vertebrate neuronal nAChRs,[85] is a potent agonist in *Ascaris* muscle[83] but without effects at the cockroach central neurone D_f.[86] Neurotoxin binding is also indicative of significant differences. In insects nAChRs are blocked by α- and K-BGT, whereas the *Ascaris* nAChR is only blocked by K-BGT. Neosurugatoxin is active in both species.[10] The exciting discovery that paraherquamide (Fig. 2), a very efficient anthelmintic natural product from *Penicillium* spp.,[87] displaced imidacloprid and α-BGT at rather low concentrations,[88] demonstrates



A



B



C

Plate 2. Schematic drawing of nAChR complex with binding sites for antiparasitic drugs. In **A** the pentameric subunit model of nAChRs (vertebrates) is shown. Different types have been described (vertebrate muscle, α_2, β, γ, δ; vertebrate neuronal, α_2, β_3) so far, in addition to several isoforms (vertebrate neuronal, $\alpha_{2\text{-}7}$, $\beta_{2\text{-}6}$.)[164] The relationship of invertebrate nAChRs to vertebrate receptors is not fully understood, but it seems that the α-subunit always participates in agonist binding. In **B** the α-subunit is shown schematically, and regions of effector binding indicated. Important sequences for IMI and agonist binding are indicated by bold lines and amino acid residues by torpedo nomenclature. α-BGT sensitivity is dependent on residues surrounding cystein 192/193.[80, 165] It is interesting that in a α-BGT weakly susceptible *C. elegans* (α-deg3) residues 189 and 198 are exchanged. In **C** an overview of the channel protein is given, showing the agonist binding site, the channel pore and an antagonistic toxin in the correct proportions.

that ligands of nAChRs, in nematodes can well lead to relevant in-vivo activities. Similar results for some of the nAChR effectors were observed by Liu and Casida.[77] Referring to electrophysiological and binding data from *Ascaris*[62,79] the order of potency for agonists was: DMPP ≈ pyrantel ≈ levamisole > ACh ≈ anatoxin ≈ nicotine ≫ cytisine ≫ IMI (own results) and for antagonists: mecamylamine ≈ *N*-methyllycaconitine ≈ α-BGT ≈ D-tubocurain ≫ decamethonium ≫ hexamethonium ≈ dihydro-β-erythroidine. Oxotremorine and 4-DAMP were of particular interest as tickicides. The presence of the neurotransmitter acetylcholine in ticks has been inferred from the susceptibility of ticks to organophosphate acaricides as well as from the demonstrated presence of acetylcholinesterase and choline acetyltransferase in *B. microplus*. However, no direct binding studies of radiolabelled nAChR effectors have been so far reported for ticks. From injection studies with isoosmotic solutions of cholinomimetics and sympathomimetics into the hemolymph of *Amblyomma hebraeum* Koch to provoke salivary fluid secretion, it was concluded that the gland itself appeared to be innervated by at least two sensory pathways, one cholinergic[89] and another perhaps catecholaminergic.[90] It was suggested that pilocarpine elicits salivation by interacting with a muscarinic type of cholinergic receptor. Since chloronicotinyls display a moderate biological activity against ticks and nematodes this gives hope that binding sites with at least some common basic features exist in AChRs of these parasites, which should allow the exploitation of chloronicotinyls as broad-spectrum parasiticides.

5.3.2 Muscarinic–cholinergic receptors

Whereas insecticides affecting nAChRs have already been identified and marketed, compounds acting on mAChRs have yet to be commercialized. Putative muscarinic responses such as binding of QNB (quinuclidinyl benzilate) and scopolamine have already been demonstrated in acarids,[91] insects[92] and nematodes.[93] Ligand binding studies showed that, in contrast to the vertebrate CNS, putative mAChRs of invertebrates are generally present at significantly lower densities ranging from 10 to 150 fmol mg^{-1} protein.[94] Among invertebrates, insects have been most intensively investigated. The cloned *Drosophila* mAChR structurally resembles best the vertebrate M_3 type; when expressed in *Xenopus* oocytes the pharmacological properties were most similar to M_1 or M_3 vertebrate subtypes.[95] This is in accordance with results obtained for honey bee, housefly and cockroach brain.[92] It was suggested that these receptor types are located postsynaptically, whereas presynaptic mAChRs resemble the vertebrate M_2 subtype, although, as for nearly all neurotransmitter receptors in insects, they do not strictly conform to the binding characteristics of the vertebrate types. When the displacement of [^{3}H]QNB was studied in the bulb mite *Rhizoglyphus echinopus* Fum. & Rob.,[91] pirenzepine, a selective antagonist of mammalian M_1 subtype, was about 1000 times more effective than the M_2-antagonist methoctramine. This is possibly indicative of putative mAChRs of the M_1 subtype. In nematodes the presence of mAChRs was also demonstrated by binding studies using [^{3}H]QNB and [^{3}H]*N*-methylscopolamine.[93] Interestingly, the concentration of [^{3}H]QNB binding sites was 3- to 4-fold higher in young L_1 larvae than in adults, which might reflect a role of mAChRs in development processes, in addition to its function in nervous tissue. Similar conclusions can be drawn from investigations on mAChRs in the epithelial cell line of *Chironomus tentans* Walk.[96] With regard to the application of muscarinic drugs as tickicides, it was shown that oxotremorine and 1-(4-dimethylaminobut-2-ynyl) pyrrolidin-2-one were the most effective in a series of muscarinic agonists against *B. microplus*.[97] Interestingly, it was demonstrated for bulb mites that the formamidine SN 49844 (amitraz metabolite) also displaced [^{3}H]QNB to some extent.[91] In view of the data available on mAChRs in parasites, it can be assumed that structural differences between vertebrate and invertebrate mAChRs might be exploited for the development of selective parasiticides.

5.4 Target sites related to parasite growth regulation

Owing to the large inherent differences between arthropod and nematode development compared to vertebrates, insect growth regulators (IGRs) are to a large extent fulfilling the requirements of being highly selective and displaying low vertebrate toxicity.[98,99] The relatively slow onset of activity restricts the application exclusively to those target pests in which a slow reduction of their populations can be tolerated. Otherwise, IGRs have to be combined with adulticides to achieve immediate protection (knock-down). Fortunately, with regard to animal health, an interesting proportion of the parasite-induced diseases is caused by different developmental stages of parasites, an example being involvement of the myiasis flies, as well as bot and warble flies. But, as inhibitors of development, IGRs may also be applied in those cases where combatting of larval and/or nymphal parasite populations would lead to a direct and significant reduction of disease-causing adults. This particularly concerns fleas, lice, mosquitoes, one-host ticks, house flies and, to a large extent, nematodes. So far, IGRs can be tentatively divided into three different categories according to their mode of action: (1) hormone mimics or anti-hormones (e.g. ecdysteroid and juvenile hormone mimics and inhibitors of their synthesis and degradation); (2) inhibitors of cuticle synthesis and degradation (chitin synthesis and chitinase inhibitors, anti-sclerotization agents) and (3) others with, so far, unclear modes of action (e.g. triazines).

5.4.1 Hormone mimics and anti-hormones (juvenoids and ecdysteroids)

The post-embryonic development of insects comprises an orderly series of stages, in the course of which they become transformed from larva into an adult or imago. The process involves growth by means of a series of moults and a metamorphosis in which the larval characteristics are lost and adult ones are differentiated. Whereas ecdysteroids stimulate the moulting process, irrespective of whether the moult leads to another larval or a pupal stage or to the imago, juvenile hormones (JHs) suppress imaginal differentiation by promoting the synthesis of larval structures. Like JHs, JH mimics and ecdysteroids also display pleiotropic actions. Various effects ranging from larvicidal and ovicidal actions to disturbances of metamorphosis, diapause and reproduction have been observed.[100] This has led to various approaches using synthetic hormone mimics as growth regulators in crop protection and animal health.

The decline in JH titre, and its clearance during the last larval instar, leads to the onset of the events associated with the metamorphosis of insect larvae into pupae. JH mediates its activity *via* binding to receptors, the JH titre being regulated both by degradation and biosynthesis. This roughly outlines the possibilities of interfering with JH action in order to achieve anti-parasitic effects. The development of appropriate synthetic juvenoids such as methoprene, fenoxycarb and pyriproxyfen have led to compounds which are reasonably stable under field conditions and which can be applied in the field of animal health and hygiene with great success against fleas, mosquitoes, cockroaches and some fly species, particularly house flies and tsetse flies.[101,102] Various attempts have been made to induce juvenoid effects in parasites, either by application of JH mimics or by inhibition of enzymes such as JH esterases[100] and/or JH epoxidases.[103] So far, only JH mimics have been applied successfully, not only in terms of biological activity, but also as far as consumer safety, toxicology and environmental protection are concerned.[100] Industrial research activities seem to have decreased in this field, even if new compounds have been developed for special purposes from time to time.[98] Major drawbacks of 'projuvenoid' action are the possible occurrence of giant larvae[99] and the relatively slow onset of action. Therefore JH antagonistic action, either as a result of interfering with hormone biosynthesis or by designing appropriate receptor ligands, would also be an interesting target for antiparasitic compounds. Another advantage of anti-juvenile hormone agents (AJHA) can, in principle, be parasite control during almost the whole period of development,[104] but the practical application of AJHAs has remained an unfulfilled prospect so far. This holds true particularly for the precocenes and some other AJHAs which failed as useful field control agents due to the high doses required and to the finding that most holometabolous insects are not susceptible to precocenes.[99] In addition, precocenes display an alkylating potential which might raise problems from a toxicological point of view. Some evidence has accumulated that JHs are also synthesized in ticks and that specific JH esterases and JH epoxide hydrolases exist in these parasites, as was demonstrated for JH III in the american dog tick, *Dermacentor variabilis* Say.[103] This is also indicative of the viability of JHs as a target for antiparasitic drugs. A new lead structure not suffering from the drawbacks of the already existing AJHAs would certainly be a stimulating breakthrough in the field of antiparasitic juvenoids.

With respect to the search for other endocrine control mechanisms of parasites, the discovery of the first synthetic ecdysteroid agoinst, tebufenozide,[12] has renewed the interest in moulting hormones as antiparasitic drugs. This compound may not only induce premature moulting but also act on ovarian development and oviposition of insects.[105] In addition, at very high concentrations, ecdysteroids have been shown to display deleterious effects on helminths.[106] Prerequisite for a further exploitation of an ecdysteroid receptor (EcR) as target is a detailed knowledge of its structure, as well as its regulatory and physiological functions. The functional receptor consists of the true ecdysteroid receptor (EcR) unit and the ultraspiracle protein (USP).[107] The heterodimeric complex binds to ecdysteroid-responsive elements on the genome which finally leads, after modulation of transcription of various hormone-dependent genes, to the physiological and morphological restructuring of the cells and tissues associated with the moulting process. Protein-binding ligands such as thyroid hormone, retinoid or vitamin D3, also belong to this group of transcription factors.[108] Receptors of this family contain ligand- and DNA-binding domains. Interestingly, the ligand-binding domain of the 9-*cis*-retinoic acid receptor-2 (RXR-α)[109] is similar to the USP. Due to the similarity of RXRs to USP, substitution of USP in functional nuclear receptor complexes is possible.[108] EcRs are expressed in various tissues and different isoforms have been identified[110] indicative of the complex regulatory hierarchy within which ecdysteroids are involved. In addition, EcRs might induce the synthesis of further transcription factors in a time- and tissue-specific pattern, leading to a highly complex regulatory hierarchy which results in the observed pleiotropic effects. The availability of EcR clones, as well as responsive DNA elements (EcRE), offers the opportunity to establish a 'reporter gene approach'. Transformed cell lines expressing high contents of EcRs, and also containing a reporter gene (e.g. luciferase) coupled to the EcRE, could easily be used to screen a high number of compounds of varied origin for their hormonal effects. Indeed, this approach was described recently in a patent application by Sumitomo Chemical Ltd.[111]

In addition to ecdysteroid effector mechanisms, steroid metabolism in insects and nematodes represents

a unique area, since neither nematodes nor insects can carry out the *de-novo* synthesis of sterols from small molecules. Both genera rely on dietary sterols for the production of moulting hormones.[99] It is hoped that disruption of uptake, transport or metabolism of dietary sterols can lead to new specific parasiticides. In contrast to phytophagous insects or plant-parasitic nematodes for which cholesterol is not available in the natural diet, vertebrate parasites take up cholesterol principally from host tissues as well as host body fluids. Thus, in arthropods, blocking any of the several biosynthetic steps between cholesterol and ecdysone would be an interesting approach, whereas in plant parasites inhibitors of dealkylation reactions leading from C_{28} and C_{29} phytosterols to cholesterol might also be exploited.[112] However, the role of steroid metabolism in both groups of parasites is surely more complicated than might be expected solely from their ecobiology. Inhibitors of the Δ^{24}-sterol reductase, such as azasteroids or alkyl amines, such as *N,N*-dimethyldodecanamine, were active not only against *M. sexta* and a number of other phytophagous insects but also against the rat parasitic nematode *Nippostrongylus brasiliensis* Lane and the cattle stomach worm *Ostertagia ostertagi* Stiles.[112] With regard to the inhibition of ecdysteroid metabolism, some inhibitors were reported to display their activity through the inhibition of cytochrome P-450-dependent ecdysone 20-monooxygenase. Plumbagin, the fungicide fenarimol and azadirachtins belong to this group.[112] The main mode of action for azadirachtin, however, is believed to be the depression of the release of prothoracicotropic hormone from the brain.[113] Although this approach has not yet led to commercially successful compounds, the investigation of cytochrome P-450-dependent oxidation reactions in particular, might lead to the discovery of new, highly active growth regulators.

5.4.2 The cuticle as a target for parasiticides

The exoskeleton of arthropods consists of different layers, of which typically the procuticle contains chitin, a major component of insect cuticle. Chitin is an amino sugar polysaccharide which is connected to proteins *via* diphenol crosslinks to form a protective matrix, comprising a chitin-microfibres-protein complex. Any interaction with the synthesis or deposition of chitin offers a potential means to control parasite development. Whereas the chitin content in insect larval cuticle amounts to 30–60%, in ticks only 3% of the cuticle weight is accounted for by chitin.[114] The pathway of arthropod chitin biosynthesis is still a matter of debate. At the present time the working hypothesis postulates pathways involving UDP-*N*-acetylglucosamine and dolichyl-*P*-*P*-$(GlcNac)_2$ [115] and/or glycolipids as intermediates during chitin synthesis (Plate 3). But the fact that some other cellular reactions, such as vesicular transport,[116] occur during biosynthesis of chitin in arthropods might also be of significance. This would lead to the speculation that GTP-binding proteins in intracellular membrane traffic (Rab/Rabphilin like proteins),[117] as well as fusion proteins, attachment proteins and their receptors,[118] might play a significant role in chitin synthesis and could perhaps be exploited as new target sites. In addition, chitin synthase activity might be superimposed by a direct regulation through phosphorylation[119] or proteolytic activation of a chitin synthase zymogen, and through indirect effects such as precursor transport and hormonal effects.[120,121] The impact of these considerations leads to the conclusion that chitin synthesis has by no means been fully exploited as an antiparasitic target, and that chances exist to discover further new drugs which cover a much wider range than just direct 'substrate inhibitors'. A hypothetical scheme of cuticle biosynthesis by epidermal cells (EC) as well as sclerotization is proposed in Plate 3 which also indicates sites of interference of various inhibitors with cuticle formation:

Trehalose, the major sugar found in the hemolymph of insects, is transported into the epidermal cells (BM, basal membrane; CM, cell membrane) and UDP-GlcNAc (Uridyl-diphospho-*N*-acetylglucosamine) is formed *via* different intermediates by various enzymes and co-factors (>). Glucose (Glc), glucose-1-phosphate (Glc-1-P), fructose-6-phosphate (Fru-6-P), glucosamine-6-phosphate (GlcN-6-P), *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P), *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P) are involved during the synthesis of UDP-GlcNAc. Chitin precursors are synthesized in excretory vesicles (exVs; perhaps of endoplasmic reticulum (ER) and golgi (GA) origin)[116,122] which, after further elongation to yield the typical microfibres and fusion with the outer cell membrane, form the chitinous network and, together with proteins and phenols, lead to tanning and hardening of the cuticle in the sclerotization process.[114] During chitin synthesis, it is proposed that chain initiation and chain elongation are taking place in two closely related but independent steps.[115] Chain initiation, which can be inhibited by tunicamycin (TM), requires as primers the synthesis of amino sugar (S) lipid respectively dolichyl-phosphate intermediates (ASLDPI), synthesized by an UDP-α-GlcNAc : dolichyl-P-GlcNAc-1-P transferase (UDT).[115,120,123,124] Only short chains are formed during this process, containing up to eight monomers of GlcNAc.[124] The ASLDPIs so formed carry the monomers in β-anomeric configuration. Long chitin chains could be formed either by assembly of such short segments or by an elongation reaction using short chains, coupled to a 'carrier', as primers. If short segments are combined during synthesis, this would lead to an α-D-'inter-segment' linkage, if a single displacement mechanism of chitin synthase is assumed. Otherwise one has to postulate a double displacement mechanism to retain the anomeric configuration. At least in fungal systems there exist no indications that chitin synthase displays

the latter property. A chitin chain would result from this process, consisting of short segments of β1-4 configuration, interrupted by α-anomeric linkages, which is not likely to display the typical, highly protective arthropod chitin layers, in which the microfibrils are arranged in parallel orientation and are stabilized by hydrogen bonds. Assuming that a 'primer-extension' reaction is more likely, the question arises which primer carrier is used for the elongation. Reports exists on the occurrence of 'chito-proteins' (CP)[125,126] which are assumed to play a role during chitin synthesis. This has not been demonstrated convincingly so far, but one can speculate that these proteins could serve as acceptors for the primer from ASLDPIs. Chitin synthase could then use these complexes in a second step for further elongation reactions, which are sensitive to nikkomycin and polyoxin inhibition.[120] From an energy consumption point of view, it is of no benefit for the organism to actively transport UDP-GlcNAc through the vesicle membrane for chitin synthesis. It is more likely that at least the chain initiation and initiation of the elongation reactions take place on the cytoplasmic side of the vesicles. This would consequently demand a translocation into the inner space of the vesicle. Perhaps chitin synthase itself (in a complex with 'chito-proteins'?) facilitates such a postulated translocation reaction. In addition, the activity of chitin synthase can be affected by various intracellular modulators. For example, the enzyme is active only in its dephosphorylated stage.[119] The chitin vesicles now have to fuse with the epidermal cell membrane, and microfibres of the final chain length have to be synthesised in order to accomplish the excretion of chitin. The mechanisms involved with this final step, and the proper arrangement of microfibres in the cuticle are unclear, but 'exocytosis-like' processes are certainly involved. These processes are regulated by G-proteins, which, after binding to GTP, stimulate Ca^{2+} uptake and mediate the fusion process.[117,118] Various modes of actions of BPUs as inhibitors of chitin biosynthesis have been proposed.[127] Interestingly, as far as excretory vesicles are concerned, diflubenzuron was shown to inhibit γ-S-GTP-stimulated uptake of calcium into vesicles.[128] Triflumuron, another benzoyl phenylurea (BPU), influenced γ-S-GTP-stimulated chitin synthesis in *Chironomus* cells,[120] indicating that BPUs might exert their activity at the level of vesicle transport and membrane fusion. Several targeting proteins (TPs), primarily during the exocytosis of neurotransmitters, have been described.[129] It is too early to speculate which or if TPs might be involved in chitin synthesis, but perhaps totally different lead structures are imaginable taking into account that specific exocytosis inhibitors are an interesting target for growth regulators.

The same is true for the degradation process catalyzed by chitinases, which were shown to be inhibited by the potent inhibitor allosamidin. Chitinases are by no means relevant only for arthropod chitin metabolism; they were also reported to play a role in nematodes,[130] indicating that this is a target with a broad antiparasitic potential.

As mentioned previously, chitin is crosslinked in the cuticle to proteins *via* diphenols. In has been shown that compounds which interfere with the biosynthesis of these sclerotization agents lead to mortality at the larval or pupal stage. The inhibition of DOPA decarboxylase (one of the important enzymes besides phenoloxidases and quinone methide isomerase) by carbidopa and DL-α-methyl-DOPA killed *L. cuprina* larvae effectively.[131] Most likely, the pupae were no longer able to control water loss sufficiently.[132] Although the mode of action of another growth regulator, cyromazine, which is predominantly active against dipteran larvae, is not known exactly, evidence has been presented that its target must be definitively different from that with BPUs[98] or nucleoside antibiotics. Other investigations involving an antioxidant, MON 0585[113] and cyromazine[134] have indicated that interference with sclerotization can well lead to interesting antiparasitic effects (Plate 3).

5.4.3 Peptide hormones as antiparasitic targets

Various approaches to interference with the action of peptide hormones in parasites are imaginable.[135] Synthetic peptides which interfere with the biological action of endogenous hormones would seem possible, but severe problems involving stability, ability to penetrate the cuticle and transport to the target site, absorption, price of synthetic pesticides, as well as homology with vertebrate peptide hormones, are to be expected.[99] Baculoviruses, as vectors carrying genes coding for antihormones, hormone-degrading enzymes or peptide hormones seem feasible,[136] but are highly speculative at the moment and far away from practical application, at least for animal health applications. Significant progress has been achieved in the identification of peptide hormones in invertebrates, mainly in arthropods, as far as parasites are concerned.[137] With regard to the use of neuropeptides as antiparasitic drugs, it was impressively demonstrated that the application of FMRF amide (H-Phe-Met-Arg-Phe-NH_2) led to strong effects on nematode (*A. suum*, *H. contortus*, *C. elegans*) muscles.[138] Interference with a mechanism common to peptide hormones of parasites from different genera would be a suitable target for the development of antiparasitic drugs (Plate 4). Nearly all secreted neuropeptides are synthesized as components of larger precursors from which active, mature molecules are cleaved, processed and modified.[139] In particular, processing by proteolytic cleavage could contribute to the specificity of neuropeptide expression and may provide a useful target for new antiparasitic drugs. This also accounts partially for the degradation of neuropeptides by membrane-bound peptidases with a relatively wide substrate specificity. Thus, specific degradation of neuro-

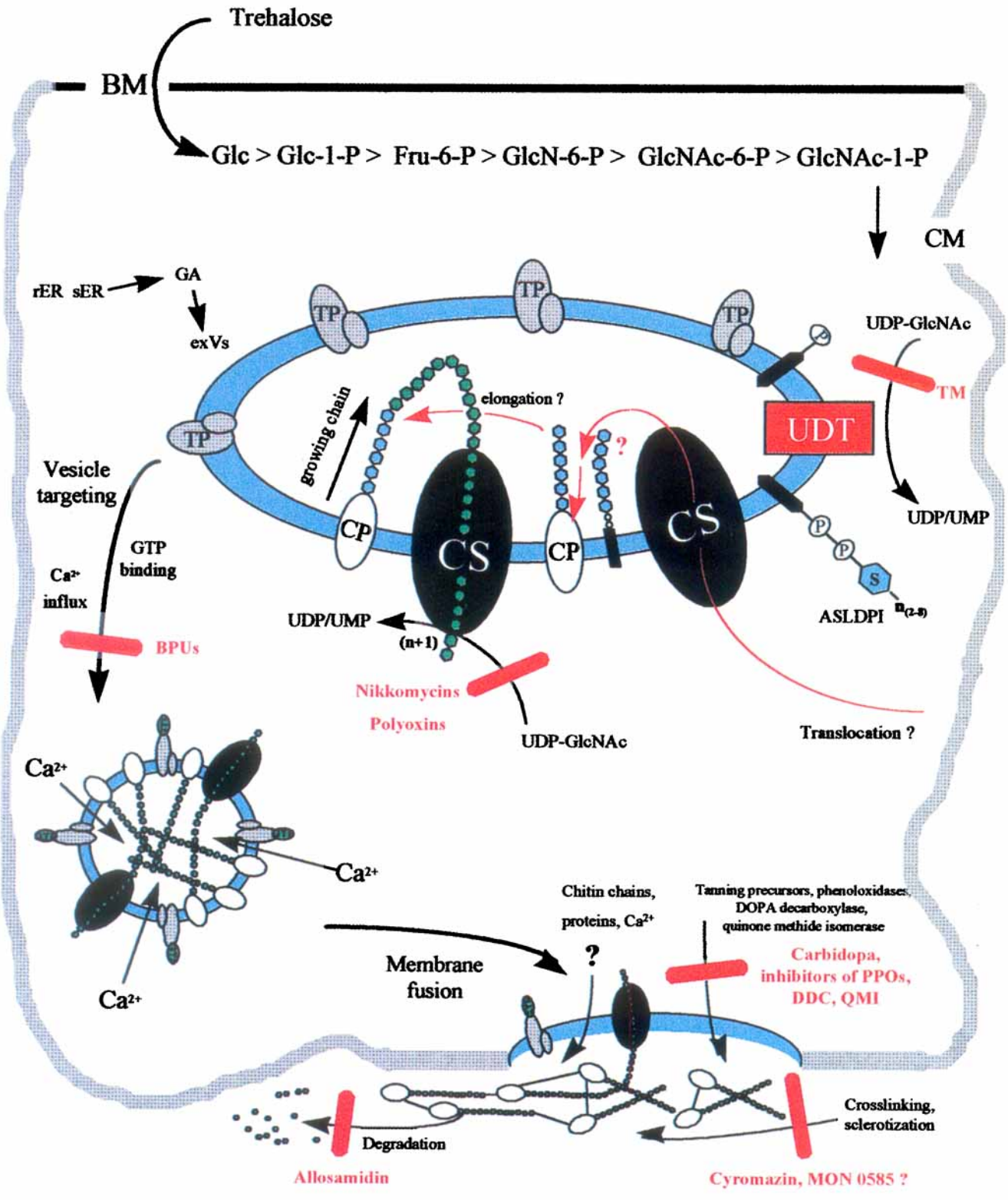


Plate 3. Schematic drawing of possible target sites of putative growth regulators. A hypothetical scheme of cuticle biosynthesis by epidermal cells (EC) as well as sclerotization is proposed. Possible sites of interference of various inhibitors of cuticle formation are indicated.

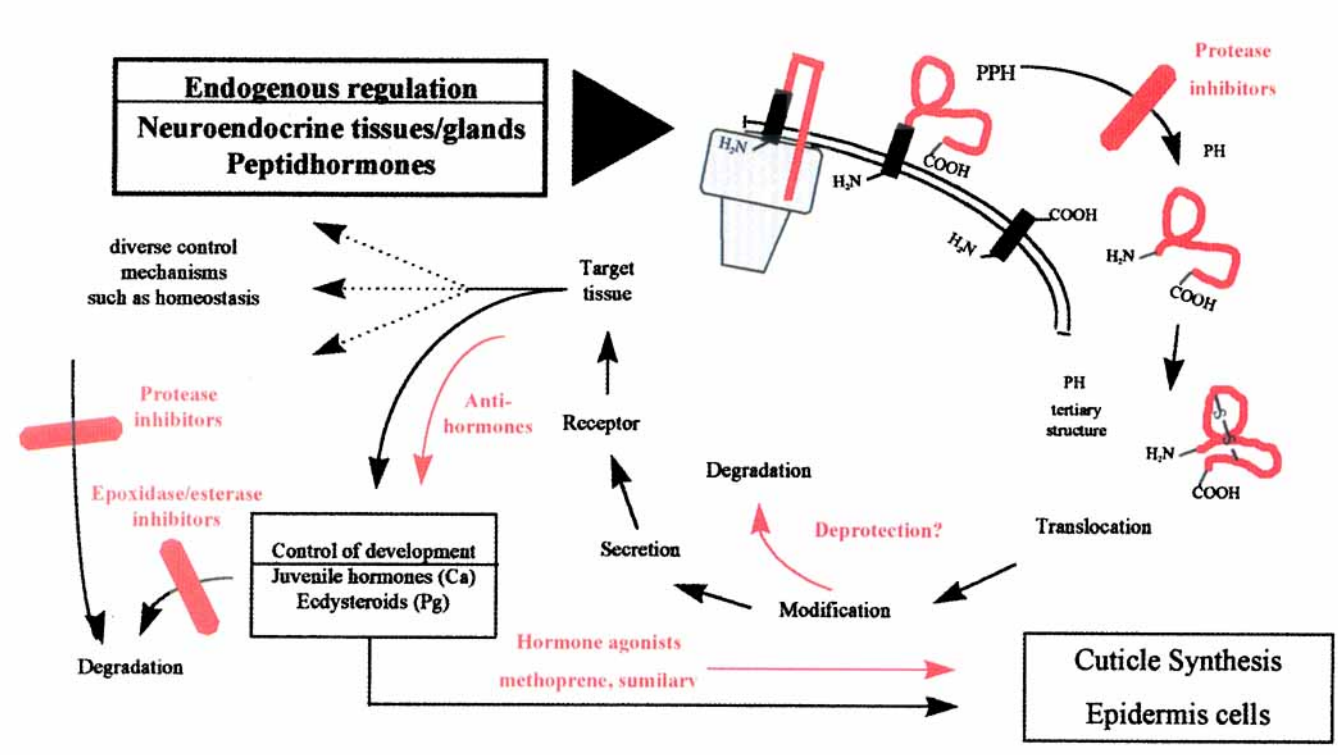


Plate 4. Schematic drawing of possible target sites interfering with hormonal regulation. The transformation of pre-prohormones (PPH) to prohormones (PH) represents the first post-translational modification of neuropeptides. Other post-translational modifications include formation of disulfide bridges (intra- and inter-hormonal), modification of N- and C-terminal amino acids (pyroglutamate and amino acid amide). To yield the prohormone, the pre-propeptide is cleaved by a signal peptidase. Active peptides are formed by prohormone convertases, which endoproteolytically cleave the prohormone.
Neuropeptides mediate various processes important for the control of homeostasis which involves, for example, the control of cuticle formation. The synthesis and secretion of hormones such as ecdysteroids and JHs are also regulated by peptide hormones. For example, ecdysteroids (prothoracic gland, Pg) regulate chitin systhesis via nuclear receptors and transcription factors which lead to the expression of a set of enzymes and proteins necessary for chitin synthesis.[99] Neuropeptides are generally degraded by amino- or carboxy-peptidases. N- and/or C-termini are rendered inactive by the action of endopeptidases which create fragments without biological activity. Possible sites of interference by protease inhibitors are indicated by bold breaks.

peptides seems to be a function of the specific tissue distribution of the involved peptidases, rather than specific cleavage sites.[140] In order to establish suitable screening systems for the search for, and design of, antiparasitic protease inhibitors, key peptidases from relevant parasites have to be isolated, cloned and expressed in advance. The prohormone convertases PC1/PC3, PC2 and PC4, all members of the subtilisin-like serine protease family[141] are particularly useful candidates for this approach, since they are expressed exclusively in endocrine or neuroendocrine tissue.[141] Among other invertebrates, subtilisin-like serine proteases have been identified e.g. in *C. elegans* (furin-like enzyme bli-4)[142] and *D. melanogaster* (fur-like genes).[143] The first insect PC2-like endoprotease has been identified very recently in *L. cuprina* using an approach based on polymerase chain reactions with two pairs of degenerated oligonucleotides located in the highly conserved catalytic domain of subtilisin-like serine proteases (Mentrup, B., Weidemann, W., Spindler, K.-D., Londershausen, M. & Turberg, A., 1995, unpublished). This offers the opportunity to use the enormous knowledge on protease inhibitors existing in pharmaceutical companies, such as the inhibition of angiotensin I-converting enzyme by captopril, as an aid to pesticide research. Since proteases also play an important role during infection with various nematodes, protease inhibitors may also well be an attractive approach for new drugs in this field. Indeed, metalloproteases,[144] serine proteases,[145] trypsin inhibitors,[146] cysteine proteases[147] and others, are of importance during the development of, and for tissue penetration in, *Ancylostoma*, *Anisakis*, *Ascaris* and *Haemonchus*. It is also interesting to note that the proteases and protease inhibitors can serve as antigens for antiparasitic vaccination approaches.

6 CONCLUDING REMARKS

The animal health industry still relies heavily on chemical agents of whatever origin for the control of parasitic diseases mediated by arthropods and helminths. So far, compounds suffer from serious drawbacks such as development of resistance (e.g. organophosphates, pyrethroids, benzimidazoles) or residues in the environment (e.g. lindane). These problems underline the need for new solutions, which will be the outcome of combined efforts in the fields of new synthetic agents or new fermentation products, and the application of bio- and 'soft'-technologies.

Biorational design of new substances will accompany this development, but, for the time being, it cannot substitute for sophisticated random screening systems. The target tissue to which the most commercially successful insecticides have been directed was, and still is, the nervous system. The number of neuroactive target sites is remarkably small, namely one enzyme (acetyl cholinesterase) and a few receptor or channel proteins (voltage- and ligand-gated sodium and chloride channels, octopamine receptor). This reflects that only few suitable deadly targets exist and/or that our drug research programmes rely on screening methods which preferentially recognize compound affecting these targets. In the context of the public demand for alternative, safe technologies this has led to the search for additional, highly selective modes of action. Growth regulators, semiochemicals or compounds which interfere specifically with the action of neuropeptides are interesting candidates for this approach.

However, in order to provide a broad information base for the identification of new lead compounds, an appropriate screening philosophy should not be restricted to either conventional live-target tests or biochemical/molecular biology assays (BMBAs) alone. To maximize the hit rate, these 'try and see' approaches should combine the relevant and cheap target parasite tests with high throughput (test capacity $\geqslant 20\,000$ compounds per year) BMBAs. This allows the exploitation of the advantages of the 'black box'-living parasite, containing all known and unknown target sites, as well as binding data gained from the receptors or enzymes under investigation, without any interference by metabolism or penetration barriers. The author envisages both approaches much more as complementary 'running mates' than competitors. Obviously, in this concept the appropriate selection of BMBAs is of major importance, but fortunately, a rather limited number of suitable target sites appears to exist.

In particular, high throughput systems (HTS) rely on large compound libraries displaying structural diversity. With regard to synthetic drugs, this is less easy to achieve than one might expect, since often only a large number of derivatives of few basically active structures is available. Libraries containing even complex mixtures of various natural compounds or derivatives from combinatorial chemistry are therefore suited for HTS, particularly when considering that, generally, toxic substances such as antimycins and others do not interfere with the target site-specific activity.

With regard to new therapeutic agents, the author is convinced that the design of innovative screening systems based on the current knowledge of parasite neurotransmitters, neuropeptides, signalling mechanisms and growth regulation, will significantly enhance the chances of discovering new drugs.

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REFERENCES

1. Kunz, S. E. & Kemp, D. H., Insecticides and acaricides: resistance and environmental impact. *Rev. Sci. Tech. Off. Int. Epiz.*, **13** (1994) 1249–86.
2. Prichard, R., Anthelmintic resistance. *Vet. Parasitol.*, **54** (1994) 259–68.
3. Naumann, K., Neue Insekizide. *Nachr. Chem. Tech. Lab.*, **42** (1994) 255–61.
4. Voss, G. & Neumann, R., Invertebrate neuroscience and its potential contribution to insect control. In Neurotox '91. *Molecular Basis of Drug and Pesticide Action*, ed. I. R. Duce. Elsevier Applied Science, London, New York, 1992, pp. VII–XX.
5. Menn, J. J., Contemporary frontiers in chemical pesticide research. *J. Agric. Food Chem.*, **28** (1980) 2–8.
6. Omura, S., Trends in the search for bioactive microbial metabolites. *J. Ind. Microbiol.*, **10** (1992) 135–56.
7. Leicht, W., Imidacloprid—a chloronicotinyl insecticide. *Pestic. Outlook*, **4** (1993) 17–21.
8. ffrench-Constant, R. H., Mortlack, D. P., Shaffer, C. D., MacIntyre, R. J. & Roush, R. T., Molecular cloning and transformation of cyclodiene resistance in *Drosophila*: An invertebrate γ-aminobutyric acid subtype A receptor locus. *Proc. Natl Acad. Sci. USA*, **88** (1991) 7209–13.
9. Trimmer, B. A., Current excitement from insect muscarinic receptors. *Trends Neurosci.*, **18** (1995) 104–11.
10. Tornoe, C., Bai, D., Holden-Dye, L., Abramson, S. N. & Sattelle, D. B., Actions of neurotoxins (bungarotoxins, neosurugatoxin and lophotoxins) on insect and nematode nicotinic acetylcholine receptors. *Toxicon*, **33** (1995) 411–24.
11. Kramer, K. J., Corpuz, L., Choi, H. K. & Muthukrishnan, S., Sequence of a cDNA and expression of the gene encoding epidermal and gut chitinases of *Manduca sexta*. *Insect Biochem. Molec. Biol.*, **23** (1993) 691–701.
12. Wing, K. D., RH 5849, a nonsteroidal ecdysone agonist: effects on a Drosophila cell line. *Science (Washington)* **241** (1988) 467–9.
13. Takeshima, H., Nishi, M., Iwabe, N., Miyata, T., Hosoya, T., Masai, I. & Hotta, Y., Isolation and characterization of a gene for a ryanodine receptor/calcium release channel in *Drosophila melanogaster*. *FEBS Lett.*, **337** (1994) 81–7.
14. Cully, D. F., Vassiliatis, D. K., Liu, K. K., Paress, P. S., Van der Ploeg, L. H. T., Schaeffer, J. M. & Arena, J. P., Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature (London)* **371** (1994) 707–11.
15. Haekler, R. E., Suhr, R. G., Sheets, J. J., Hatton, C. J., Johnson, P. L., Davis, L. N., Edie, R. G., Kaster, S. V., Jourdan, G. P., Jackson, J. L. & Krumkalus, E. V., Chemistry and miticidal activity of fused pyrimidine derivatives of fenazaquin. In *Advances in the Chemistry of Insect Control III*, ed. G. G. Briggs. Royal Society of Chemistry, Cambridge, 1994, pp. 70–84.
16. Hunt, D. A., 2-Arylpyrroles: novel uncouplers of oxidative phosphorylation. In *Advances in the Chemistry of Insect Control III*, ed. G. G. Briggs. Royal Society of Chemistry, Cambridge, 1994, pp. 127–40.
17. Schaeffer, J. M., Blizzard, T. A., Ondeyka, J. G., Goegelman, R., Sinclair, P. J. & Mrozik, H., [^{3}H]Paraherquamide binding to *Caenorhabditis elegans*: studies on a potent new anthelmintic agent. *Biochem. Pharm.*, **43** (1992) 679–84.
18. Sasaki, T., Takagi, M., Yaguchi, T., Miyadoh, S., Okada, T. & Koyama, Y., A new anthelmintic cyclodepsipeptide, PF1022 A. *J. Antibiot.*, **45** (1992) 692–7.
19. Shoop, W. L., Mrozik, H. & Fisher, M. H., Structure and activity of avermectins and milbemycins in animal health. *Vet. Parasitol.*, **59** (1995) 139–56.
20. Tanaka, Y. & Omura, S., Agroactive compounds of microbial origin. *Ann. Rev. Microbiol.*, **47** (1993) 57–87.
21. Hall, L. M., Ren, D., Feng, G., Eberl, D. F., Dubald, M., Yang, M., Hannan, F., Kousky, C. T. & Zheng, W., Calcium channel as a new potential target for insecticides. In *Molecular Action of Insecticides on Ion Channels*, ed. J. M. Clark. ACS Symposium Series, 1995, 591, pp. 162–72.
22. Fleckenstein, A., Specific pharmacology of calcium in myocardium, cardiac peacemakers and vascular smooth muscle. *Ann. Rev. Pharmacol. Toxicol.*, **17** (1977) 149–66.
23. Clark, M. J., Ion-channel diversity—Present and future roles in insecticide action. In *Molecular Action of Insecticides on Ion Channels*, ed. J. M. Clark. ACS Symposium Series, 1995, 591, pp. 1–23.
24. Striessnig, J., Berger, W. & Glossmann H., Molecular properties of voltage-dependent Ca^{2+} channels in excitable tissues. *Cell Physiol. Biochem.*, **3** (1993) 295–317.
25. Lehmberg, E. & Casida, J. E., Similarity of insect and mammalian ryanodine binding sites. *Pestic. Biochem. Physiol.*, **48** (1994) 145–52.
26. Pelzer, S., Barhanin, J., Pauron, P., Trautwein, W., Lazdunski, M. & Pelzer, D., Diversity and novel pharmacological properties of Ca^{2+} channels in *Drosophila* brain membranes. *EMBO J.*, **8** (1989) 2365–71.
27. Pessah, I. N., Chemical effectors of the ryanodine receptor: a novel strategy for insect control. In *Recent Advances in the Chemistry of Insect Control*, ed. L. Crombie. Royal Society of Chemistry, Special Publication, RSC Cambridge, No. 79, 1989, pp. 278–96.
28. Waterhouse, A. L., Pessah, I. N., Francini, A. O. & Casida, J. E., Structural aspects of ryanodine action and selectivity. *J. Med. Chem.*, **30** (1987) 710–6.
29. Mack, M. M., Molinski, T. F., Buck, E. D. & Pessah, I. N., Novel modulators of skeletal muscle FKBP12/calcium channel complex from *Ianthella basta*. *J. Biol. Chem.*, **269** (1994) 23236–49.
30. Smith, D. R., The development of Tickgard. A commercial vaccine against the cattle tick *Boophilus microplus*. In *8th Internat. Cong. Parasitology*, Izmir, Turkey, ed. M. A. Oezcel & M. Z. Alkan, Abstracts 1, 1994, Abstract 26.1, 580, p. 98.
31. Payne, C. C., Microbial control of insect pests: current and potential uses. *Chem. Ind.* (1989) 182–6.
32. Gupta, R. K. & Rutledge, L. C., Role of repellents in vector control and disease prevention. *Am. J. Trop. Med. Hyg.*, **50** (1994) 82–6.
33. Moore, W. S., Profita, J. C. & Koehler, C. S., Soaps for home landscape insect control. *Calif. Agric.*, **6** (1979) 13–5.
34. Jones, O. T., The current use of pheromones and other semiochemicals in the integrated management of insect pests. *Semiochem. Pestic. Outlook*, **8** (1994) 26–31.
35. Bjostad, L. B., Hibbard, B. E. & Cranshaw, W. S., Application of semiochemicals in integrated pest management programs. In *Pest Control with Enhanced Environmental Safety*, ed. S. O. Duke, J. J. Menn & J. R. Plimmer. ACS Symposium Series, 524, 1993, pp. 199–218.
36. Jones, O. T., Monitoring and control of insect pests with pheromones and other semiochemicals. *Argo-Industr. Hi-Tech.*, **2** (1991) 27–32.
37. Faulde, M., Fuchs, M. E. & Nagel, W., Sex-, stadium- and stress-specific analysis of the salivary proteins of the german cockroach, *Blattella germanica L.*. *J. Insect Physiol.*, **36** (1990a) 41–50.

38. Faulde, M., Fuchs, M. E. & Nagel, W., Further characterization of a dispersion-inducing contact pheromone in the saliva of the german cockroach, *Blattella germanica L.* (Blattodae: Blattellidae). *J. Insect Physiol.*, **36** (1990b) 353–9.
39. Davis, E. E., Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). *J. Med. Entomol.*, **22** (1985) 237–43.
40. Rao, S. S., Rao, K. M. & Ramachandran, P. K., Chemistry, efficacy and toxicology of insect repellents, *J. Sci. Ind. Res.*, **47** (1988) 722–35.
41. Kumar, S., Prakash, S., Kaushik, M. P. & Rao, K. M., Comparative activity of three repellents against the ticks *Rhipicephalus sanguineus* and *Argas persicus. Med. Vet. Entom.*, **6** (1992) 47–50.
42. Schreck, C. E., Fish, D. & McGovern, T. P., Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). *J. Am. Mos. Contr. Ass.*, **11** (1995) 136–40.
43. Robert, L. L., Hallam, J. A., Seeley, D. C., Roberts, L. W. & Wirtz, R. A., Comparative sensitivity of four *Anopheles* (Diptera: Culicidae) to five repellents. *J. Med. Entom.*, **28** (1991) 417–20.
44. Boeckh, J., Breer, H., Geier, M., Hoever, F. P., Krüger, B. W., Nentwig, G. & Sass, H., Acylated 1,3-aminopropanols as repellents against blood sucking arthropods. *Pestic. Sci.*, **48** (1996) 359–73.
45. Ashworth, J. R. & Wall, R., Responses of the sheep blowflies *Lucilia sericata* and *L. cuprina* to odour and the development of semiochemical baits. *Med. Vet. Entomol.*, **8** (1994) 303–9.
46. Stetter, J., Schallner, O., Wroblowsky, M. I., Behrenz, W., Stendel, W. & Becker, B. (Bayer AG), *European Pat.*, No. 201852, 1985.
47. Buntain, I. G., Hatton, L. R., Hawkins, D. W., Pearson, C. J. & Roberts, D. A. (May and Baker), *European Pat.*, No. 295117, 1987.
48. Bloomquist, J. R., Cyclodiene resistance at the insect GABA receptor/chloride channel complex confers broad cross resistance to convulsants and experimental phenylpyrazole insecticides. *Arch. Insect Biochem. Physiol.*, **26** (1994) 69–79.
49. Liu, M. Y. & Casida, J. E., High affinity binding of [^{3}H]imidacloprid in the insect acetylcholine receptor, *Pestic. Biochem. Physiol.*, **40** (1993) 40–6.
50. Geary, T. G., Klein, R. P., Vancover, L., Bowman, J. W. & Thompson, D. P., The nervous system of helminths as targets for drugs. *J. Parasitol.*, **78** (1992) 215–30.
51. Rauh, J. J., Lummis, S. C. R. & Sattelle, D. B., Pharmacological and biochemical properties of insect GABA receptors. *Trends Pharmacol. Sci.*, **11** (1990) 325–9.
52. Johnson, C. D. & Stretton, A. O. W., GABA-immunoreactivity in inhibitory motor neurons of the nematode. *Ascaris. J. Neurosci.*, **7** (1987) 223–35.
53. Stephenson, F. A., The $GABA_A$ receptors. *Biochem. J.*, **310** (1995) 1–9.
54. Bai, D. & Sattelle, D. B., A $GABA_B$ receptor on an identified insect motor neurone. *J. Exp. Biol.*, **198** (1995) 889–94.
55. Martin, R. J., Pennington, A. J., Duittoz, A. H., Robertson, S. & Kusel, J. R., The physiology and pharmacology of neuromuscular-transmission in the nematode parasite *Ascaris suum. Parasitol.*, **102** (1991) 41–58.
56. Sattelle, D. B., GABA receptors of insects. *Adv. Insect Physiol.*, **22** (1990) 1–113.
57. Waldrop, B. & Hildebrand, J. G., GABA mediated synaptic inhibition of projection neurons in the antennal lobes of the sphinx moth, *Manduca sexta. J. Comp. Physiol.*, **161** (1987) 23–32.
58. Holden-Dye, L., Krogsgaard-Larsen, P., Nielsen, L. & Walker, R. J., GABA receptors on the somatic muscle cells of the parasitic nematode, *Ascaris suum*: stereoselectivity indicates similarity to a $GABA_A$-type agonist recognition site. *Br. J. Pharmacol.*, **98** (1989) 841–50.
59. Buckingham, S. D., Hosie, A. M., Roush, R. L. & Sattelle, D. B., Actions of antagonists and convulsant antagonists on a *Drosophila melanogaster* GABA receptor (Rdl) homo-oligomer expressed in *Xenopus* oocytes. *Neurosci. Lett.*, **181** (1994) 137–40.
60. Colliot, F., Kukorowski, K. A., Hawkins, D. W. & Roberts, D. A., Fipronil: a new soil and foliar broad spectrum insecticide. In *Brighton Crop Prot. Conf.—Pests and Diseases*, 1992, pp. 29–34.
61. Liu, M.-Y., Lanford, J. & Casida, J. E., Relevance of [^{3}H]imidacloprid binding site in house fly head acetylcholine receptor to insecticidal activity of 2-nitromethylene- and 2-nitroimino-imidazolidines. *Pestic. Biochem. Physiol.*, **46** (1993) 200–6.
62. Walker, R. J., Colquhoun, L. M., Parri, H. R., Williams, R. G. & Holden-Dye, L., Pharmacology of the *Ascaris* nervous system. In Neurotox '91. *Molecular Basis of Drug and Pesticide Action*, ed. I. R. Duce. Elsevier Applied Science, London, New York, 1992, **8**, pp. 105–21.
63. Laughton, D. L., Amar, M., Thomas, P., Towner, P., Harris, P., Lunt, G. G. & Wolstenholme, A. J., Cloning of a putative inhibitory amino acid receptor subunit from the parasitic nematode *Haemonchus contortus. Receptors Chann.*, **2** (1994) 155–63.
64. ffrench-Constant, R. H., Cloning of the *Drosophila* cyclodiene insecticide resistance gene: A novel $GABA_A$ receptor subtype? *Comp. Biochem. Physiol.*, **104C** (1993) 9–12.
65. Terada, M., Neuropharmacological mechanism of action of PF 1022 A, an antinematode anthelmintic with a new structure of cyclic depsipeptide, on *Angiostrongylus cantonensis* and isolated frog rectus, *Jpn. J. Parasitol.*, **41** (1992) 108–17.
66. Martin, R. J., Harder, A., Londershausen, M. & Jeschke, P., Anthelmintic actions of the cyclic depsipeptide PF1022A and electrophysiological effects on muscle cells of *Ascaris suum. Pestic. Sci.*, **48** (1996) 343–9.
67. Geßner, G., Meder, S., Rink, T., Boheim, G., Harder, A., Jeschke, P., Scherkenbeck, J. & Londershausen, M., Ionophorous and anthelmintic activity of PF1022A, a cyclooctadepsipeptide, are not related. *Pestic. Sci.*, **48** (1996) 399–407.
68. Pleiß, U., Harder, A., Turberg, A., Londershausen, M., Iinuma, K., Mencke, N., Jeschke, P. & Bonse, G., Synthesis of radiolabeled cyclodepsipeptide [^{3}H]-methyl PF1022A. *J. Label. Comp. Radiopharm.*, **38** (1995) 61–9.
69. Chen, W., Terada, M. & Cheng, J. T., Characterization of subtypes of gamma-aminobutyric acid receptors in an *Ascaris* muscle preparation by binding assay and binding of PF1022A, a new anthelmintic, on the receptors. *Parasitol. Res.*, **82** (1996) 97–101.
70. Eldefrawi, M. E., Abalis, I. M., Filbin, M. T. & Eldefrawi, A. T., Glutamate and GABA receptors of insect muscles: biochemical identification and interactions with insecticides. In *Approaches to New Leads for Insecticides*, ed. H. C. von Keyserlingk, A. Jäger & Ch. von Szczepunski. Springer Verlag, Berlin, 1985, pp. 101–16.
71. Schroeder, M. E. & Flattum, R. F., The mode of action and neurotoxic properties of the nitromethylene heterocycle insecticides. *Pestic. Biochem. Physiol.*, **22** (1984) 148–60.

72. Nishimura, K., Kanda, Y., Okazawa, A. & Ueno, T., Relationship between insecticidal and neurophysiological activities of imidacloprid and related compounds. *Pestic. Biochem. Physiol.*, **50** (1994) 51–9.
73. Leech, C. A., Jewess, P., Marshall, J. & Sattelle, D. B., Nitromethylene actions on *in-situ* and expressed insect nicotinic acetylcholine receptors. *FEBS Letters*, **290** (1991) 90–4.
74. Orr, G. L., Orr, N. & Hollingworth, R. M., Localization and pharmacological characterization of nicotinic-cholinergic binding sites in cockroach brain using α- and neuronal bungarotoxin. *Insect Biochem.*, **20** (1990) 557–66.
75. Badio, B. & Daly, J. W., Epibatidine, a potent analgetic and nicotinic agonist. *Molec. Pharm.*, **45** (1994) 563–9.
76. Yamamoto, I., Yabuta, G., Tomizawa, M., Saito, T., Miyamoto, T. & Kagabu, S., Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. *Nihon Noyaku Gakkaishi* (*J. Pestic. Sci.*), **20** (1995) 33–40.
77. Liu, M. Y. & Casida, J. E., High affinity binding of [^{3}H]imidacloprid in the insect acetylcholine receptor, *Pestic. Biochem. Physiol.*, **40** (1993) 40–6.
78. Tomizawa, M., Otsuka, H., Miyamoto, T. & Yamamoto, I., Pharmacological effects of imidacloprid and its related compounds on the nicotinic acetylcholine receptor with its ion channel from the *Torpedo* electric organ. *Nihon Noyaku Gakkaishi* (*J. Pestic. Sci.*), **20** (1995) 49–56.
79. Harrow, I. D. & Gration, A. F., Mode of action of the anthelmintics morantel, pyrantel and levamisole on muscle cell membrane of the nematode *Ascaris suum*. *Pestic. Sci.*, **16** (1985) 662–72.
80. Tomlinson, G., Albuquerque, C. A. & Woods, R. A., The effects of amidantel (Bay d 8815) and its deacylated derivative (Bay d 9216) on *Caenorhabditis elegans*. *Eur. J. Pharm.*, **113** (1985) 255–62.
81. Pinnock, R. D., Sattelle, D. B., Gration, K. A. F. & Harrow, I. D., Actions of potent cholinergic anthelmintics (morantel, pyrantel and levamisole) on an identified insect neurone reveal pharmacological differences between nematode and insect acetylcholine receptors. *Neuropharmacology*, **27** (1988) 843–8.
82. Sattelle, D. B., Pinnock, R. D. & Lummis, S. C. R., Voltage-independent block of a neuronal nicotinic acetylcholine receptor by *N*-methyllycaconitine. *J. Exp. Biol.*, **142** (1989) 215–25.
83. Colquhoun, L., Holden-Dye, L. & Walker, R. J., The pharmacology of cholinoreceptors on the somatic muscle cells of the parasitic nematode *Ascaris suum*. *J. Exp. Biol.*, **158** (1991) 509–30.
84. Trimmer, B. A. & Weeks, J. C., Effects of nicotinic and muscarinic agents on an identified motoneurone and its direct afferent inputs in larval *Manduca sexta*. *J. Exp. Biol.*, **144** (1989) 303–37.
85. Bertrand, D., Ballivet, M. & Rungger, D., Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes. *Proc. Natl Acad. Sci. USA*, **87** (1990) 1993–7.
86. David, J. A. & Sattelle, D. B., Actions of cholinergic pharmacological agents on cell body membrane of fast coxal depressor motoneurone of the cockroach (*Periplaneta americana*). *J. Exp. Biol.*, **108** (1984) 119–36.
87. Ondeyka, J. G., Goegelman, R. T., Schaeffer, J. M., Keleman, L. & Zitano, L., Novel antinematodal and antiparasitic agents from *Penicillium charlessii*. I. Fermentation, isolation and biological activity. *J. Antibiot.*, **43** (1990) 1375–9.
88. Blythe, J., Cutler, P., Dubar, S. & Earley, F., Novel pharmacology of insect neuronal nicotinic receptor. Conference Poster, Zeneca, *Neuronal Nicotinic Receptors, INCERM*, Straßburg, 16–20. Oct. 1994.
89. Kaufman, W. R., Aeschlimann, A. A. & Diehl, P. A., Regulation of body volume by salivation in a tick challenged with fluid loads. *Am. J. Physiol.*, **238** (1980) 102–12.
90. Kaufman, W. R., Actions of some transmitters and their antagonists on salivary secretion in a tick. *Am. J. Physiol.*, **235** (1987) 76–81.
91. Huang, Z. & Knowles, C. O., Properties of a quinuclidinyl benzilate binding component in the bulb mite. *Comp. Biochem. Physiol.*, **95C** (1990) 71–7.
92. Abdallah, E. A. M., Eldefrawi, M. E. & Eldefrawi, A. T., Pharmacologic characterization of muscarinic receptors of insect brains. *Insect Biochem. Physiol.*, **17** (1991) 107–18.
93. Culotti, J. G. & Klein, W. L., Occurence of muscarinic acetylcholine receptors in wild type and cholinergic mutants of *Caenorhabditis elegans*. *J. Neurosci.*, **3** (1983) 359–68.
94. Breer, H., Receptors for acetylcholine in the nervous system of insects. In *Neurotox '88, Molecular Basis of Drug & Pesticide Action*, ed. G. G. Lunt. Excerpta Medica, Amsterdam, New York, 1988, **22**, pp. 301–9.
95. Blake, A. D., Anthony, N. M., Chen, H. H., Harrison, J. B., Nathanson, N. M. & Sattelle, D. B., *Drosophila* nervous system muscarinic acetylcholine receptor: transient functional expression and localization by immunocytochemistry. *Molec. Pharmacol.*, **44** (1993) 716–24.
96. Lammerding-Koeppel, M., Spindler-Barth, M. & Drews, U., 20-OH-ecdysone-induced morphogenetic movements in a *Chironomus* cell line are accompanied by expression of an embryonic muscarinic system. *Roux's Arch. Dev. Biol.*, **203** (1994) 439–44.
97. Bigg, D. C. H., & Purvis, S. R., Muscarinic agonists provide a new class of acaricide. *Nature* (*London*), **262** (1976) 220–2.
98. Graf, J.-F., The role of insect growth regulators in arthropod control. *Parasitol. Today*, **9** (1993) 471–4.
99. Spindler-Barth, M., Endocrine strategies for the control of ectoparasites and insect pests. *Parasitol. Res.*, **78** (1992) 89–95.
100. Hammock, B. D. & Quistad, G. B., Metabolism and mode of action of juvenile hormone, juvenoids, and other insect growth regulators. In *Progress in Pesticide Biochemistry*, ed. D. H. Hutson & T. R. Roberts. John Wiley & Sons, Ltd, 1981, **1**, pp 1–83.
101. Londershausen, M., Alig, B., Pospischil, R. & Turberg, A., Activity of novel juvenoids on arthropods of veterinary importance. *Arch. Insect Biochem. Physiol.*, **32** (1996) 651–8.
102. Henrick, C. A., Juvenile hormone analogs: structure–activity relationships. In *Insecticide Mode of Action*, ed. J. R. Coats. Academic Press, New York, London, 1982, pp. 315–402.
103. Roe, R. M., Kallapur, V. L., Majumder, C., Lassiter, M. T., Apperson, C. S., Sonenshine, D. E. & Winder, B. S., Biochemical evidence for the presence of a juvenoid in ticks. In *Host Regulated Developmental Mechanisms in Vector Arthropods.*, ed. D. Borovsky & A. Spielman. Florida Press IFAS, Vero Beach, 1993, pp. 110–9.
104. Staal, G. B., Anti juvenile hormone agents. *Ann. Rev. Entomol.*, **31** (1986) 391–429.
105. Wing, K. D. & Aller, H. E., Ecdysteroid agonists as novel insect growth regulators. In *Pesticides and Alternatives: Innovative Chemical and Biological Approaches to Pest Control*, ed. J. E. Casida. Elsevier, Amsterdam, 1990, pp. 251–7.

106. Spindler, K.-D., Spindler-Barth, M. & Mehlhorn, H., Effects of the juvenile hormone antagonist precocene II and the moulting hormone 20-OH-ecdycone on *Litosomoides carinii* and *Dipetalonema viteae in vitro*. *Z. Parasitenkd.*, **72** (1986) 837–41.
107. Yao, T. P., Forman, B. M., Jiang, Z., Cherbas, L., Chen, J.-D., McKeown, M., Cherbas, P. & Evans, R. M., Functional ecdysone receptor is the product of EcR and ultraspiracle genes. *Nature (London)*, **366** (1993) 476–9.
108. Segraves, W. A., Steroid receptors and orphan receptors in *Drosophila* development. *Sem. Cell Biol.*, **5** (1994) 105–13.
109. Bourget, W., Ruff, M., Chambon, P., Gronemeyer, H. & Moras, D., Crystal structure of the ligand-binding of the human nuclear receptor RXR-α. *Nature (London)*, **375** (1995) 377–82.
110. Talbot, W. S., Swyryd, E. A. & Hogness, D. S., *Drosophila* tissues with different metamorphic response to ecdysone express different ecdysone receptor items. *Cell*, **73** (1993) 1323–37.
111. Mikya, K. & Myakudu, M., Plasmid for the determination of moulting hormone activity comprising the 20-hydroxy-ecdysone reporter gene, heat shock promoter and luciferase gene. *Japanese Patent* No. 06335390-A, 1993.
112. Svoboda, J. A. & Chitwood, D. I., Inhibition of sterol metabolism in insects and nematodes. In *Regulation of Isopentoid Metabolism*, ed. W. D. Nes, E. J. Parish & J. M. Trzaskos. ACS Symposium Series 497, 1992, pp. 205–18.
113. Koolmann, J., Bidmon, H. J., Lehmann, M. & Käuser, G., On the mode of action of azadirachtin in blowfly larvae and pupae. In *Endocrinological Frontiers in Physiological Insect Ecology.*, ed. F. Sehnal, A. Zabza & D. L. Denlinger. Wrodov Technical University Press, Wodrov, 1988, pp. 55–67.
114. Peter, M. G., Die molekulare Architektur des Exoskeletts von Insekten. *Chemie unserer Zeit*, **27** (1993) 189–97.
115. Spindler-Barth, M., Spindler, K.-D., Londershausen, M. & Thomas, H., Inhibition of chitin synthesis in an insect cell line. *Pestic. Sci.*, **25** (1989) 115–21.
116. Zimowska, G., Mikalajczyk, P., Silhacek, D. L. & Oberlander, H., Chitin synthesis in *Spodoptera frugiperda* wing imaginal discs. II. Selective action of chlorfluazuron on wheat germ agglutinin binding and cuticle ultrastructure. *Arch. Insect. Biochem. Physiol.*, **27** (1994) 89–108.
117. Fischer von Mollard, G., Stahl, B., Li, C., Südhof, T. C. & Jahn, R., Rab proteins in regulated exocytosis. *Trends Biochem. Sci.*, **19** (1994) 164–8.
118. Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E., SNAP receptors implicated in vesicle targeting and fusion. *Nature (London)*, **362** (1993) 318–24.
119. Ishii, S. & Matsumura, F., Diflubenzuron-induced changes in activities of cAMP-dependent protein kinase in the newly molted integument of the american cockroach *in situ* and in cell-free conditions. *Insect Biochem. Molec. Biol.*, **22** (1992) 69–72.
120. Londershausen, M., Turberg, A., Ludwig, M., Hirsch, B. & Spindler-Barth, M., Properties of insect chitin synthase: effects of inhibitors, γ-S-GTP and compounds influencing membrane lipids. In *Chitin and Chitosan*, 1996, accepted.
121. Spindler, K.-D., Spindler-Barth, M. & Londershausen, M., Chitin metabolism: a target for drugs against parasites. *Parasitol. Res.*, **76** (1990) 283–8.
122. Horst, M. & Walker, A., Crustacean chitin synthesis and the role of the Golgi apparatus—*in vivo* and *in-vitro* studies. In *International Symposium on Chitin Enzymology*, ed. A. A. Muzzarelli, Senigallia, 1993, p. 19.
123. Horst, M. N., Molecular and cellular aspects of chitin synthesis in larval *Artemia*. In *Cell and Molecular Biology of Artemia Development*, ed. A. H. Warner, T. H. McRae & J. C. Bagshaw. Plenum Press, New York, 1989, pp. 59–76.
124. Horst, M. N., The biosynthesis of crustacean chitin. Isolation and characterization of polyprenol-linked intermediates from Brine Shrimp microsomes. *Arch. Biochem. Biophys.*, **223** (1983) 254–63.
125. Porcheron, P., Moriniere, M., Condouel, N. & Oberlander, H., Ecdysteroid-stimulated synthesis and secretion of a *N*-acetyl-D-glucosamine-rich glycopeptide in a lepidopteran cell line derived from imaginal discs. *Arch. Insect Biochem. Physiol.*, **16** (1991) 257–71.
126. Kramerov, A. A. Mukha, D. V., Metakovsky, E. V. & Gvozdev, V. A., Glycoproteins containing sulfated chitin-like carbohydrate moiety are synthesized in an established *Drosophila melanogaster* cell line. *Insect Biochem.*, **16** (1986) 417–32.
127. Grosscurt, A. C. & Jongsma, B., Mode of action and insecticidal properties of diflubenzuron. In *Chitin and Benzoylphenyl Ureas*, ed. J. E. Wright & A. Retnakaran. Dr W. Junk Publishers, Dordrecht, Boston, 1987, **4**, pp. 75–99.
128. Nakagawa, Y. & Matsumura, F., Diflubenzuron affects gamma-thio GTP-stimulated Ca^{2+} transport *in-vitro* in intracellular vesicles from the integument of the newly molted american cockroach, *Periplaneta americana L. Insect Biochem. Molec. Biol.*, **24** (1994) 1009–15.
129. Monck, J. R., Oberhauser, A. F., Robinson, I. M. & Fernandez, J. M., Neurotransmitter release through the exocytotic fusion pore. *Sem. Neurosci.*, **6** (1994) 177–85.
130. Cohen, E., Chitin synthesis and degradation as targets for pesticide action. *Arch. Insect Biochem. Physiol.*, **22** (1993) 245–61.
131. Turnbull, I. F. & Howells, A. J., Larvicidal activity of inhibitors of DOPA decarboxylase on the Australian sheep blowfly, *Lucilia cuprina. Aus. J. Biol. Sci.*, **33** (1980) 169–81.
132. Turnbull, I. F., Pyliotis, N. A. & Howells, A. J., The effects of DOPA decarboxylase inhibitors on the permeability and ultrastructure of the larval cuticle of the Australian sheep blowfly, *Lucilia cuprina. J. Insect Physiol.*, **26** (1980) 525–32.
133. Walton, B. T., Sanborn, J. R. & Metcalf, R. L., Structure–activity relationships for insect growth regulators derived from substituted di-*tert*-butyl phenols. *Pestic. Biochem. Physiol.*, **12** (1979) 23–30.
134. Binnington, K. C., Ultrastructural changes in the cuticle of the sheep blowfly, *Lucilia* induced by certain insecticides and biological inhibitors. *Tissue Cell*, **17** (1985) 131–40.
135. Menn, J. J. & Borkovec, A. B., Insect neuropeptides: potential new insect control agents. *J. Agric. Food Chem.*, **37** (1989) 271–8.
136. Masler, E. P., Kelly, T. J. & Menn, J. J., Insect neuropeptides: discovery and application in insect management. *Arch. Insect Biochem. Physiol.*, **22** (1993) 87–111.
137. Nachman, R. J., Holman, G. M. & Haddon, W. F., Leads for insect neuropeptide mimetic development. *Arch. Insect Biochem. Physiol.*, **22** (1993) 181–97.
138. Thompson, D. P., Maule, A. G., Bowman, J. W., Davis, J. P., Winterrowd, C. A., Larsen, M. J., Friedman, A. R., Klein, R. D. & Geary T. G., Rational approaches to anthelmintic discovery. In *Novel Approaches to the Control of Helminth Parasites of Livestock*, Armidale,

Australia, Conference Abstracts, 1995, p. 40.
139. O'Shea, M., Introduction to neuropeptides: perspectives for the parasitologist. *Parasitology*, **102** (1991) 71–5.
140. Turner, A. I., Hooper, N. M. & Kenny, A. J., Neuropeptide-degrading enzymes. In *Neuropeptides: A Methodology*, ed. G. Fink & A. J. Harman. John Wiley & Sons, Chichester, UK, 1989, pp. 189–223.
141. Birch, N. P., Hakes, D. I., Dixon, J. E. & Mezey, E., Distribution and regulation of the candidate prohormone processing enzymes SPC2 and SPC3 in adult brain. *Neuropeptides*, **27** (1994) 307–22.
142. Peters, K. & Rose, A., Bil-4 encodes a protein structurally related to the yeast serine endoprotease Kex2. *Worm Breeders Gazette*, **11** (1991) 28.
143. Roebroek, A. I. M., Ayoubi, T. A. Y., Creemers, J. W. M., Pauli I. G. L. & van de Ven, W. J. M., The Dfur2 gene of *Drosophila melanogaster:* genetic organization, expression during embryogenesis and pro-protein processing activity of its translational product Dfurin2. *DNA Cell Biol.*, **14** (1995) 223–34.
144. Hawdon, J. M., Jones, B. F., Perregaux, M. A. & Hotez, P. J., *Ancylostoma caninum* alloprotease release coincides with activation of infective larvae *in vitro*. *Exp. Parasitol.*, **80** (1995) 205–11.
145. Morris, S. R. & Sakanari, J. A., Characterization of the serine protease and serine protease inhibitor from the tissue-penetrating nematode *Anisakis simplex*. *J. Biol. Chem.*, **269** (1994) 27650–6.
146. Hawley, J. H. & Peanasky, R. J., *Ascaris suum:* are trypsin inhibitors involved in species specificity of Ascarid nematodes?. *Exp. Parasitol.*, **75** (1992) 112–18.
147. Pratt, D., Armes, L. G., Hageman, R., Reynolds, V., Boisvenue, R. J. & Cox, G. N., Cloning and sequence comparisons of four distinct cysteine proteases expressed by *Haemonchus contortus* adult worms. *Molec. Biochem. Parasitol.*, **51** (1992) 209–18.
148. Kanbe, K., Takahashi, A., Tamamura, T. & Sato, K., Isolation and structures of two novel anti-mite substances, AB3217-B and C. *J. Antibiotics*, **45** (1992) 368–9.
149. Wang, M. T., Quin, H. L., Kong, M. & Ki, Y. Z., Insecticidal sesquiterpene polyol ester from *Celastrus angulatus*. *Phytochemistry*, **30** (1991) 3931–3.
150. Sakuda, S., Isogai, A., Matsumoto, A., Suzuki, A. & Koseki, K., The structure of allosamidin, a novel insect chitinase inhibitor, produced by *Streptomyces sp.*, *Tetrahedron Lett.*, **27** (1986) 2475–8.
151. Takahashi, A., Kurasawa, S., Ikeda, D., Okami, Y. & Takeuchi, T., Altemicidin, a new acaricidal and antitumor substance. I. Taxonomy, fermentation, isolation and physicochemical and biological properties. *J. Antibiot.*, **42** (1989) 1556–66.
152. Londershausen, M., Leicht, W., Lieb, F., Moeschler, H. & Weiss, H., Molecular mode of action of annonins. *Pestic. Sci.*, **33** (1991) 427–38.
153. Takahashi, A., Saito, N., Hotta, K., Okami, Y. & Umezawa, H., Bagougeramines A and B, new nucleoside antibiotics produced by a strain of *Bacillus circulans*. I. Taxonomy of the producing organism and isolation and biological properties of the antibiotics. *J. Antibiot.*, **39** (1986) 1033–46.
154. Schaeffer, J. M., Frazier, E. G., Bergstrom, A. R., Williamson, J. M. & Liesch, J. M., Cochlioquinone A, a nematocidal agent which competes for specific [^{3}H]ivermectin binding sites, *J. Antibiot.*, **43** (1990) 1179–82.
155. Imamura, N., Kuga, H., Otoguro, K., Tanaka, H. & Omura, S., Structures of jietacins: unique α,β-unsaturated azoxy antibiotics. *J. Antibiot.*, **42** (1989) 156–8.
156. Isogai, A., Sakuda, S., Matsumoto, S., Ogura, M., Furihata, K., Seto, H. & Suzuki, A., The structure of leucanicidin, a novel insecticide produced by *Streptomyces halstedii*. *Agric. Biol. Chem.*, **48** (1984) 1379–81.
157. Hayashi, H., Takiuchi, K., Murao, S. & Arai, M., Structure and insecticidal activity of new indole alkaloids, okaramines A and B, from *Penicillium simplicissimum* AK-40. *Agric. Biol. Chem.*, **53** (1989) 461–9.
158. Larson, L. L., Thompson, G. D., Sparks, T., Hill, B. & Schoonover, J., Insecticidal activity of *Saccharopolyspora spinosa:* a novel actinomycete. In *ESA Annual Meeting*, Dallas, USA, 1994, AB 0849, 152.
159. Murao, S. Sakai, T., Gibo, H. Nakayama, T. & Shin, T., A novel trehalase inhibitor, trehalostatin, produced by *Amycolatopsis trehalostatica*. *Agric. Biol. Chem.*, **55** (1991) 895–7.
160. Asano, N., Takeuchi, M., Kameda, Y., Matsui, K. & Kono, Y., Trehalase inhibitors, validoxylamine A and related compounds as insecticides. *J. Antibiot.*, **43** (1990) 722–6.
161. Goetz, M. A., McCormick, P. A., Monaghan, R. L. & Ostlind, D. A., L 155,175: A new antiparasitic macrolide—fermentation, isolation and structure. *J. Antibiot.*, **38** (1985) 161–8.
162. Cully, D. F., Vassilatis, D. K., Liu, K. K., Paress, P. S., Van der Ploeg, L. H. T., Schaeffer, J. M. & Arena, J. P., Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature (London)*, **371** (1994) 707–10.
163. Rogers, E., Koniuszy, F., Shavel, J. & Folkers, K., Plant insecticides. I. ryanodine, a new alkaloid from *Ryania speciosa*. *Vahl. J. Am. Chem. Soc.*, **70** (1948) 3086–8.
164. Fleming, J. T., Tornoe, C., Riina, H. A., Coadwell, J., Lewis, J. A. & Sattelle, D. B., Acetylcholine receptor molecules of the nematode *Caenorhabditis elegans*. In *Comparative Molecular Neurobiology*, ed. Y. Pichon. Birkhäuser Verlag, Basel, 1993, pp. 65–80.
165. McLane, K. E., Wu, X. & Conti-Tronconi, B. M., Structural determinants within residues 180–199 of the rodent α_5 nicotine acetylcholine receptor subunit involved in α-bungarotoxin binding. *Biochemistry*, **30** (1991) 10730-8.